



# Proceedings of the Plant Development Workshop & the Canadian Society of Plant Physiologists

Eastern Regional Meeting

## Délibérations de le Congrès de Développement Végétale & la Société Canadienne de Physiologie Végétale

Congrès régional de l'est

December 5th-6th, 2008  
Royal Ontario Museum  
University of Toronto



Canadian Society of  
Plant Physiologists  
Société Canadienne de  
Physiologie Végétale



Ecology & Evolutionary Biology  
UNIVERSITY OF TORONTO



<http://PDW-CSPP-ERM-2008.notlong.ca>

**Welcome to the 2008  
Eastern Meeting of the Plant Development Workshop  
& the Canadian Society of Plant Physiologists**

**Bienvenue au le 2008  
Congrès de Développement Végétale  
& la Société Canadienne de Physiologie Végétale**

**Royal Ontario Museum  
University of Toronto**

**Friday December 5th - Saturday December 6th, 2008**

**Local Organizing Committee:**

Malcolm Campbell, Dept. Cell and Systems Biology, University of Toronto  
email: malcolm.campbell@utoronto.ca  
Tim Dickinson, Dept. of Natural History, Royal Ontario Museum  
email: tim.dickinson@utoronto.ca

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## PROGRAM OVERVIEW

### Friday, December 5<sup>th</sup> (Royal Ontario Museum, Level 1B, Signy & Cléopée Eaton Theatre)

- 16:30 Registration opens (Admission to the ROM will be free for registrants on Friday from this point onward)
- 19:00 Welcome (Tim Dickinson & Malcolm Campbell)
- 19:10 Keynote Seminar: Quentin Cronk (University of British Columbia)  
**"Evolution and molecular development of bird pollinated flowers"**  
 ROM, Level 1B, Signy & Cléopée Eaton Theatre
- 20:00 Gala Mixer
- 21:15 Registrants must depart ROM

### Saturday, December 6<sup>th</sup> (University of Toronto, Earth Sciences Centre, 5 Bancroft Ave)

- 08:15 Registration Opens (refreshments available)
- 08:30 Poster Setup
- 09:00 Welcome (Tim Dickinson & Malcolm Campbell)
- 09:10 Keynote Seminar: Jen Sheen (Harvard University)  
**"Plant-bacteria warfare: Innate immunity & bacterial suppressors"**
- 10:00 Poster Session I (Odd Numbered Posters)
- 10:45 Contributed Seminars Session I (Concurrent Sessions)
- 12:30 Buffet Lunch
- 12:30 CSPP/SCPV Executive Lunch
- 12:45 Poster Session II (*Even Numbered Posters*)
- 13:30 Poster Session II (*Odd Numbered Posters*)
- 14:00 Keynote Seminar: Michael Emes (University of Guelph)  
**"Making starch - a simple glucose polymer requiring complex bio chemistry"**
- 14:50 Poster Session III (refreshments provided; *Even Numbered Posters*)
- 15:25 Remove Posters
- 15:30 Contributed Seminar Session II (Concurrent Sessions)
- 17:30 Acknowledgements
- 17:45 Awards for Student Presentations  
 CSPP Regional Director's Awards for Best Student Seminar and Poster
- 18:00 Meeting Ends

## DETAILED SCIENTIFIC PROGRAM

Saturday, December 6<sup>th</sup> (University of Toronto, Earth Sciences Centre, 5 Bancroft Ave)

- 09:00 Welcome (ESC 1050)
- 09:10 Keynote Seminar: Jen Sheen (Harvard University; (ESC 1050)  
**"Plant-Bacteria Warfare - Innate Immunity & Bacterial Suppressors"**
- 10:00 Poster Session I (Odd Numbered Posters; Foyer)
- 10:45 Contributed Seminars Session I (Concurrent Sessions; 1A, 1B, 1C)  
\*denotes student presenter  
Contributed Seminar Session 1-A (ESC1050; abstracts 1A-1 to 1A-7)  
Chair: Professor Keiko Yoshioka, University of Toronto
- 10:45 1A-1 **"Abiotic stress associated divergence in gene expression following an *Arabidopsis thaliana* whole-genome duplication."**  
Gregory Downs\* and Lewis Lukens
- 11:00 1A-2 **"The *Populus* drought response is shaped by genotype and time of day"**  
Olivia Wilkins\*, Malcolm M. Campbell
- 11:15 1A-3 **"*Populus* drought transcriptome: Intraspecific differences in transcriptome activity"**  
Erin T. Hamanishi\*, Olivia Wilkins, Sherasha Raj, and Malcolm M. Campbell
- 11:30 1A-4 **"The Role of Alternative Oxidase during Low Temperature Growth in *Nicotiana tabacum*"**  
Steven (Jia) Wang\*, Sasan Amirsadeghi and Greg C. Vanlerberghe
- 11:45 1A-5 **"The Role of the Mitochondrion and Alternative Oxidase during *Pseudomonas Syringae* Infection of *Nicotiana tabacum* Leaves"**  
Marina Cvetkovska\* and Greg C. Vanlerberghe
- 12:00 1A-6 **"Photoperiodic injury in tomato is linked to diel N metabolism and mediated by protein tyrosine nitration."**  
L. Tian\*, D' Silva, B. J. Micallef, and B. Grodzinski
- 12:15 1A-7 **"Sorting of the Carnation Italian ringspot virus replication protein p36 to the mitochondrial outer membrane is mediated by an internal targeting signal and the TOM complex"**  
Yeen Ting Hwang\*, Andrew W. McCartney, Satinder K. Gidra, and Robert T. Mullen

Contributed Seminar Session 1-B (ESC142; abstracts 1B-1 to 1B-7)

Chair: Professor Barbara Moffatt, University of Waterloo

- 10:45 1B-1 **"Novel Green to Red photo-convertible EosFP based probes for plants"**  
Alison M. Sinclair, Mike Schenkel\*, Chris Trobacher, Daniel Johnstone, Devon Radford, Neeta Mathur, John Greenwood, J. Derek Bewley and Jaideep Mathur
- 11:00 1B-2 **"Peroxisome formation: An early intracellular response to specific forms of oxidative stress"**  
Alison M. Sinclair\*, Neeta Mathur, Jaideep Mathur

- 11:15 **1B-3 "The N-terminal acidic domain of the *Arabidopsis* chloroplast preprotein receptors atToc132 and atToc159 are intrinsically unstructured"**  
Lynn G.L. Richardson\*, Masoud Jelokhani-Niaraki and Matthew D. Smith
- 11:30 **1B-4 "Protein import into chloroplasts in the single-cell C4 species *Bieneria sinuspersici*: Identification of translocons at the outer envelope membrane of chloroplasts"**  
Terry S.C. Lung\*, and Simon D.X. Chuong
- 11:45 **1B-5 "The sorting of tail-anchored *Arabidopsis* OEP9, Toc33 and Toc34 is mediated by distinct targeting signals and the lipid composition of the plastid outer envelope"**  
Preetinder K. Dhanoa\*, Matthew P. Henderson, Matthew D. Smith, David W. Andrews and Robert T. Mullen
- 12:00 **1B-6 "*AtBXL1* encodes a bifunctional  $\beta$ -D-xylosidase/ $\alpha$ -L-arabinofuranosidase required for pectic arabinan modification in *Arabidopsis* mucilage secretory cells"**  
Andrej A. Arsovski\*, Theodore M. Popma, George W. Haughn, Nicholas C. Carpita, Maureen C. McCann and Tamara L. Western
- 12:15 **1B-7 "The role for *BLADE-ON-PETIOLE1* and 2 in conversion of shoots to flowers during the floral transition in *Arabidopsis*"**  
Mingli Xu\*, Tieqiang Hu and Shelley Hepworth

Contributed Seminar Session 1-C (ESC149; abstracts 1C-1 to 1C-7)

Chair: Professor Sharon Regan, Queen's University

- 10:45 **1C-1 "Soybean stem residue for auto industry"**  
Reinprecht Y., Dodds H., Ablett G., Poysa V., Rajcan I. and K. P. Pauls
- 11:00 **1C-2 "Overexpression of cytosolic glutamine synthetase in rice improves grain yield and nitrogen harvest index"**  
Liz K. Brauer\*, Amanda Rochon, Steven Rothstein, Barry J. Shelp
- 11:15 **1C-3 "Laboratory Assays Confirm Native uses of Sweet Fern (*Comptonia peregrina*) for its medicinal properties"**  
Matthew Edwards\*, Breanne Duquette, Bill Dew, Philippe Babady-Bila, Breanne Copeland, Tony Parkes and Ewa Cholewa
- 11:30 **1C-4 "Pot Size Matters"**  
P. Audet\* and C. Charest
- 11:45 **1C-5 "Flow cytometric measurements of oxidative stress in freshly isolated algal symbionts as a function of temperature and iron availability to coral colonies."**  
Iglic, K.\*, Hewlett, V. B, Shick, J. M., Wells, M. L., Trick, C. G., Dunlap, W. C.
- 12:00 **1C-6 "Investigation of the factors impacting mycorrhizal density in natural populations of *Fragaria virginiana*"**  
Ieff H. Taylor
- 12:15 **1C-7 "High stability ferric chelates result in decreased iron uptake by the green alga *Chlorella kessleri* due to decreased ferric reductase activity and chelation of ferrous iron."**  
Harold G. Weger, Jackie Lam, Nikki L. Wirtz, Crystal N. Walker and Ron G. Treble



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December 5-6, 2008

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- 12:30 Buffet Lunch
- 12:30 CSPP/SCPV Executive Lunch
- 12:45 Poster Session II (*Even Numbered Posters*; Foyer)
- 13:30 Poster Session II (*Odd Numbered Posters*; Foyer)
- 14:00 Keynote Seminar: Michael Emes (University of Guelph; ESC 1050)  
**"Making starch - a simple glucose polymer requiring complex bio chemistry"**
- 14:50 Poster Session III (refreshments provided; *Even Numbered Posters*; Foyer)
- 15:25 Remove Posters (Foyer)
- 15:30 Contributed Seminar Session II (Concurrent Sessions; 2A, 2B, 2C)  
\*denotes student presenter  
Contributed Seminar Session 2-A (ESC1050; abstracts 2A-1 to 2A-8)  
Chair: Professor Robert Mullen, University of Guelph
- 15:30 **2A-1 "CBB Resistance in *Phaseolus vulgaris*: Towards the Identification of a Resistance Gene"**  
Perry GE\*, Reinprecht Y, Chan JK and Pauls KP
- 15:45 **2A-2 "Investigation of DIR1 movement during long distance signalling in Systemic Acquired Resistance in *Arabidopsis*"**  
M. Champigny, J. Faubert, H. Shearer, P. Fobert, and R.K. Cameron
- 16:00 **2A-3 "A novel *Arabidopsis* SAR mutant, *pac2*, shows enhanced hypersensitive cell death in response to pathogen infection"**  
Huoi Ung\*, Wolfgang Moeder, and Keiko Yoshioka
- 16:15 **2A-4 "Investigation of abiotic stress responses in the pathogen resistant mutant *cpr22*"**  
Stephen Mosher\*, Wolfgang Moeder, Yusuke Jikumaru, Eiji Nambara, Keiko Yoshioka
- 16:30 **2A-5 "The *Pseudomonas syringae* Type III Effector HopF2<sub>Pto DC3000</sub> Suppresses PAMP- and Effector-Triggered Plant Immune Responses"**  
Mike Wilton\*, Gopal Subramaniam, James Elmore, Corinna Felsensteiner, Gitta Coaker, and Darrell Desveaux
- 16:45 **2A-6 "Chemical genomic investigation of the *Arabidopsis thaliana*-*Pseudomonas syringae* pathosystem"**  
Karl Schreiber\*, Wenzislava Ckurshumova, James Peek, and Darrell Desveaux
- 17:00 **2A-7 "HopZ1a: a *Pseudomonas syringae* type III effector protein recognized by the RAZ1 resistance protein of *Arabidopsis thaliana*"**  
Jennifer D. Lewis, Ronald Wu, David S. Guttman, Darrell Desveaux.
- 17:15 **2A-8 "Dissection of plant resistance to pest using a genomic approach: *Arabidopsis*-Two Spotted Spider Mite *Tetranychus urticae*, novel model for plant-herbivore interactions"**  
Vojislava Grbic, Cherise Poo and Miodrag Grbic

**Contributed Seminar Session 2-B (ESC 142; abstracts 2B-1 to 2B-8)**

Chair: Professor Shelley Hepworth, Carleton University

- 15:30    **2B-1 "Ethylene Receptors: A Wealth of Knowledge Beyond the Hypocotyl Model"**  
Jonathan M. Plett\* and Sharon Regan
- 15:45    **2B-2 "Screening a potato activation tagged mutant population identifies important developmental regulators involved in flowering and tuber development"**  
Jeremy L. Duguay\*, Vicki Gustafson, and Sharon Regan
- 16:00    **2B-3 "Regulatory roles of plant microRNAs and small interfering RNAs in *Arabidopsis thaliana*"**  
Shuhua Zhan\*, and Lewis Lukens
- 16:15    **2B-4 "AtMBD9 Modulates *Arabidopsis* Development through the Dual Epigenetic Pathways of DNA Methylation and Histone Acetylation"**  
Mahmoud W.F. Yaish, Mingsheng Peng and Steven J. Rothstein
- 16:30    **2B-5 "Analysis of *HUA2* LIKE (HULK) gene Family in *Arabidopsis thaliana*"**  
Sathya S Challa\* and Vojislava Grbic
- 16:45    **2B-6 "Heat stress induces autophagic programmed cell death of microspore mother cells in *Oryza sativa* (var. *japonica*)"**  
Shaheen S. Bagha\* and Tammy L. Sage
- 17:00    **2B-7 "Developmental Programmed Cell Death (PCD) in Lace Plant (*Aponogeton madagascariensis*)"**  
Harrison Wright, Christina E. Lord, Kendra A. Sauerteig, and Arunika N. Gunawardena
- 17:15    **2B-8 "Network Models in Auxin Signal Transduction and Vascular Tissue Patterning"**  
Thomas Berleth, Naden Krogan, Enrico Scarpella, George Stamatiou, Danielle Marcos

**Contributed Seminar Session 2-C (ESC 149; abstracts 2C-1 to 2C-8)**

Chair: Professor Norman Huner, University of Western Ontario

- 15:30    **2C-1 "Nuclear targeting of methyl recycling enzymes is mediated by a specific protein-protein interaction"**  
Sanghyun Lee\*, Andrew C. Doxey, and Barbara Moffatt
- 15:45    **2C-2 "5'-methylthioadenosine nucleosidase-deficient plants exhibit developmental abnormalities"**  
Ishari Waduware\*, Sarah Schoor, Katharina Bürstenbinder, Subhash Minocha, Margret Sauter and Barbara Moffatt
- 16:00    **2C-3 "Adenosine kinase deficiency alters cell proliferation and cytokinin profiles in *Arabidopsis thaliana*"**  
Sarah Schoor\*, Scott Farrow, Neil Emery and Barbara Moffatt
- 16:15    **2C-4 "Investigating the role of APT1 isoforms in purine salvage and subcellular localization"**  
Antonio Facciolo\* and Barbara Moffatt

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- 16:30    **2C-5 "Redox modulation of starch synthases in wheat and maize endosperm"**  
          Mark M. Burrell\*, Amina Makhmoudova, Ian J. Tetlow, Michael J. Emes
- 16:45    **2C-6 "Plants acquired essential functions and diverse mechanisms of metabolic regulation through the evolution of ancient and recent shikimate kinase gene duplicates"**  
          Geoffrey Fucile\* and Dinesh Christendat
- 17:00    **2C-7 "Taxonomic Distribution of Alternative Oxidase in Non-Angiosperm Plants"**  
          Allison E. McDonald and James F. Staples
- 17:15    **2C-8 "Regulation and localization of arogenate dehydratases in *Arabidopsis thaliana*"**  
          Zachary B. Armstrong, Oliver R.A. Corea, Rebecca L. Hood, Mark A. Bernards, and Susanne E. Kohalmi
- 17:30    Acknowledgements
- 17:45    Awards for Student Presentations  
          CSPP Regional Director's Awards for Best Student Seminar and Poster
- 18:00    Meeting Ends



## Poster Presentations

Presenting author's name underlined. \* denotes student poster

No.	Authors	Title
P1	<u>Fadi Al-Daoud*</u> , A. Mohammad, and R.K. Cameron	<b>NAC transcription factors play a number of roles during age-related resistance in <i>Arabidopsis thaliana</i>.</b>
P2	<u>R. Alhattab*</u> , B. Miki, and T. Xing	<b>Characterization of <i>Arabidopsis HD2</i> histone deacetylases in plant development.</b>
P3	<u>Muhammad Arif*</u> and Peter K. Pauls	<b>Mechanical properties of soybean protein films as affected by protein compositions.</b>
P4	C. Begy and <u>E. Cholewa</u>	<b>Mechanisms of aluminum resistance in <i>Eriophorum vaginatum</i>.</b>
P5	<u>Stacey A. Bruce*</u> and R. J. Neil Emery	<b>Production of hormones by axenic cultures of <i>Bradyrhizobium japonicum</i>, <i>Rhizobium leguminosorum</i> biovar <i>viciae</i> and <i>Rhizobium lupini</i>.</b>
P6	<u>L. Chen</u> , L. Gyenis, B. Dempsey, J. Brandle and S. Dhaubhadel	<b>Stability of IL-10 transcripts affects IL-10 protein accumulation in <i>Arabidopsis thaliana</i>.</b>
P7	<u>Claire Chesnais*</u> , Jeremy Duguay, Shawn Mansfield, Sharon Regan	<b>Linking phenotype to genotype in rosewood, an activation-tagged poplar mutant.</b>
P8	<u>L.S. Chia</u> , A. Jensen, L.C. Wright, L-T. Lim, C. Moresoli, L. Simon, R.L. Legge and K.P. Pauls	<b>Physicochemical properties of edible films from dry bean genotypes protein.</b>
P9	<u>Viktoriya Coneva*</u> , Tong Zhu, and Joseph Colasanti	<b>Transcriptome profiling suggests a link between carbon assimilation, photosynthesis and floral induction in maize.</b>
P10	<u>K. Dahal*</u> , K. Kane, F. Sarhan, B. Grodzinski and N. Hüner	<b>Cold acclimated winter cereals exhibit an enhanced potential for CO<sub>2</sub> assimilation under elevated CO<sub>2</sub> conditions.</b>
P11	<u>Thomas A. DeFalco*</u> , Brent N. Kaiser, and Wayne A. Snedden	<b>Identification and characterization of a novel calmodulin-binding kinase from soybean root nodules.</b>

P12	<u>M.R. Derynck*</u> , J. Yi and S. Dhaubhadel	Differential expression of CHS7 and CHS8 genes in soybean
P13	<u>Jennifer Drouin*</u> , Samantha Crossley, Kristan Washburn, and Ewa Cholewa	Developmental anatomy of <i>Arabidopsis thaliana</i> : creating a model for growth stages analysis through a new histochemical technique.
P14	<u>Kimberley H. Gibson*</u> , Alex S. Howard <sup>1</sup> , Satinder K. Gid <sup>1</sup> , John S. Greenwood, and Robert T. Mullen	The tomato bushy stunt virus replication proteins, p33 and p92, in concert with the host- cell ESCRT machinery, are involved in the biogenesis of peroxisome multivesicular bodies in <i>Saccharomyces cerevisiae</i> .
P15	<u>Satinder K. Gid<sup>1</sup></u> , Jay M. Shockey, John M. Dyer, and Robert T. Mullen	The type 8 and 9 glycerol 3-phosphate acyltransferase (GPAT) enzymes are localized to the same ER subdomain, but possess distinct ER retrieval motifs: functional divergence of the dilysine ER retrieval motif in plant cells.
P16	<u>Katrina E. Haasen*</u> , Yolanda T. Chong, Chris Sanford, and Daphne R. Goring	An investigation of the exocyst complex, and its role in compatible pollen-pistil interactions in <i>Arabidopsis</i> .
P17	<u>Shuxian Hiu*</u> , Ryan Austin and Nicholas Provart	Validation of <i>de novo</i> bioinformatic predictions of <i>Arabidopsis thaliana</i> cis-regulatory motifs using <i>in planta</i> GFP/GUS expression assays.
P18	<u>Christine Holley*</u> , Christopher P. Trobacher, and John S. Greenwood	SLCYSPRO, a Vacuolar Enzyme Potentially Involved in Endosperm Programmed Cell Death is Regulated by Gibberellic Acid and Ethylene.
P19	Rebecca L. Hood, Mark A. Bernards, and <u>Susanne E. Kohalmi</u>	Quantitative expression analysis for six <i>Arabidopsis</i> AROGENATE DEHYDRATASEs in response to heat and cold stress.
P20	<u>Megan A. House*</u> , Mary Ann Fieldes	Comparison of DNA methylation at different times of the day in an early-flowering line of flax ( <i>Linum usitatissimum</i> L.) and its control.
P21	<u>Aaron D. Johnstone*</u> , Shawn C. Chafe, Jacqueline B. Pierce, Robert T. Mullen, Dev Mangroo	Regulation of retrograde movement of tRNA from the cytoplasm to the nucleus by nutrient stress may not be universally conserved between yeast, mammals and plants.
P22	<u>Pragya Kant</u> , Wen-Zhe Liu, Pat Masliamany and K. Peter Pauls	Inhibition of <i>Fusarium graminearum</i> growth by corn defensin protein expressed in <i>Escherichia coli</i> and <i>Pichia pastoris</i> .

P23	Madiha Khan, Mingli Xu, Tieqiang Hu, Kate Storey, Jethro Mercado, and <u>Shelley Hepworth</u>	<b><i>BLADE-ON-PETIOLE 1</i> and <i>2</i> interact antagonistically with <i>BREVIPEDICELLUS</i> and <i>BELLRINGER</i> to control <i>Arabidopsis</i> inflorescence architecture.</b>
P24	<u>M. Krol</u> , A.G. Ivanov, E. Selstam, L. Quigley <sup>1</sup> , V. Hurry, R Gardiner, N.P.A. Huner	<b>Phosphatidylglycerol is required for chloroplast biogenesis during cold acclimation of <i>Arabidopsis thaliana</i>.</b>
P25	<u>Chloë M. Lazakis*</u> and Joseph Colasanti	<b>Investigation of a putative mobile, long-distance signal that promotes flowering in maize.</b>
P26	<u>Esther Lesmana*</u> and Dan Riggs	<b>Characterization of suppressor and enhancer mutants of <i>BREVIPEDICELLUS</i> in <i>Arabidopsis thaliana</i>.</b>
P27	<u>Matthew G. Letts</u>	<b>Quantifying the impact of slope aspect position on physiological stress in grasses and shrubs of a northern semiarid grassland during severe drought.</b>
P28	Lande Liu, Yizhizheng and <u>Beixin Mo</u>	<b>Potential roles of <i>Em (LEA1)</i> protein in the increased salt-tolerance in transgenic tobacco plant.</b>
P29	<u>Christina E. Lord*</u> , and Arunika N. Gunawardena	<b>The lace plant: transformation.</b>
P30	<u>Mitchell J.R. MacLeod*</u> , Michael D. BeGora, and Elizabeth A. Weretilnyk	<b>Molecular and phylogenetic characterization of related <i>N</i>-methyltransferases from <i>Arabidopsis thaliana</i>.</b>
P31	<u>A. Marcellus*</u> , E. Cholewa	<b><i>Eriophorum vaginatum</i>: senescence patterns within the vegetative and flowering culms.</b>
P32	<u>Beixin Mo</u> , J. Derek Bewley	<b>The utilization of stored and newly-synthesized mRNAs during seed germination.</b>
P33	<u>Michelle Moody*</u> , Annette Nassuth	<b>Identifying Vitaceae inducer of CBF expression (ICE) genes.</b>
P34	<u>Annette Nassuth</u>	<b>Identification of potential post-transcriptional modification events that regulate the grape transcriptome in response to stress.</b>

P35	<u>Nievas M. S*</u> , Wang Y., Yoshioka K. and Desveaux D.	<b>Identifying <i>Pseudomonas syringae</i> Type III effector proteins that modulate auxin signaling in <i>Arabidopsis thaliana</i>.</b>
P36	<u>Reena Pinhero</u> , A.G.Marangoni, Rickey Y Yada	<b>Alleviation of low temperature sweetening in potato by overexpressing <i>Arabidopsis thaliana</i> Pyruvate decarboxylase 1</b>
P37	<u>Michael B. Prouse*</u> , Christian Dubos, Julia M. Romano, and Malcolm M. Campbell	<b>Transcriptional regulation and downstream targets of AtMYB61.</b>
P38	<u>Ian Pulsifer*</u> , Christine Lowe, Frances Tran, Gopal Subramaniam, and Owen Rowland	<b>Differential gene expression in <i>Arabidopsis thaliana</i> in response to infection by <i>Fusarium graminearum</i>.</b>
P39	<u>Resmi N. Radhamony</u> , Jami Bryan, Linda Bourassa, Jay M. Shockey, John M. Dyer, J.M. and Robert T. Mullen	<b>Post-translational regulation of ER membrane-bound plant fatty acid desaturase enzymes.</b>
P40	<u>Sherosha Raj*</u> , Olivia Wilkins, Erin T. Hamanishi, and Malcolm M. Campbell	<b><i>Populus</i> drought transcriptome: Spatial and temporal variation in transcriptome activity.</b>
P41	<u>Riaz, M*</u> , Pauls, KP, Erikson, L and Raizada, MN	<b>Characterization of corn cellulose fiber for manufacturing automotive plastic parts</b>
P42	Dominic Rosso, <u>Rainer Bode*</u> , Wenzel Li, Diego Saccon, Shelly Wang, Lori A. Schillaci, Steven R. Rodermeier, Denis P. Maxwell, and Norman P.A. Hüner	<b>Excitation pressure controls the development of variegation in <i>Arabidopsis thaliana</i>.</b>
P43	<u>Anthony M. Silva*</u> , Melanie P. Columbus, and Daniel D. Lefebvre	<b>Phytoremediation of a chemical plume containing 1,4-dioxane.</b>
P44	<u>Michael E. Stokes*</u> , Matthew Waller, Julia Romano, Gillian Dean, George Haughn, Shawn Mansfield, Malcolm M. Campbell	<b>Dissection of the AtMYB61 regulatory circuit by chemical genetics.</b>
P45	<u>I. Szucs*</u> , M. Escobar, R. R. Cloutier, C. W. Beninger, and B. Grodzinski	<b>Within the newly re-classified Plantaginaceae are iridoids merely secondary metabolites?</b>

P46	<u>Mimi Tanimoto</u> , Reynald Tremblay and Joseph Colasanti	<b>Altered gravitropism, amyloplast sedimentation and circumnutation in <i>atidd15/sgr5</i> mutants are associated with reduced starch levels.</b>
P47	<u>Christopher P. Trobacher</u> <sup>¶</sup> , Adriano Senatore, Christine Holley, and John S. Greenwood	<b>A KDEL-tailed cysteine proteinase associated with programmed cell death in post-germinative tomato endosperm.</b>
P48	<u>Paul J. Turgeon</u> <sup>*</sup> , Rashida Patel, & Dan Riggs	<b>Ectopic Expression Of An F-Box Protein Alters Inflorescence Architecture</b>
P49	Sollapura Vishwanath, Reem Alhattab, Robin Visser, <u>Christine Lowe</u> <sup>*</sup> , Sarah Amer, Jennifer Lee, Jasmine Ono and Owen Rowland	<b>FAR4 and FAR5: Fatty acyl-CoA reductases from <i>Arabidopsis thaliana</i> that generate fatty alcohols associated with suberin deposition.</b>
P50	<u>Sarathi M. Weraduwage</u> <sup>*</sup> , Shezad A. Rauf, Malgre C. Micallef, David C. Taylor, Bernard Grodzinski and Barry J. Micallef	<b><i>Arabidopsis thaliana</i> (L.) Heynh. having altered expression of mitochondrial pyruvate dehydrogenase kinase show enhanced oil biosynthesis under elevated CO<sub>2</sub>.</b>
P51	<u>Yun-Yun Wu</u> <sup>*</sup> , Min Yu, Margie Gruber, Isobel Parkin, and Sharon Regan	<b>Activation tagging to identify new genes responsible for trichome development in <i>Arabidopsis thaliana</i>.</b>
P52	<u>Weilong Xie</u> , Youn-Seb Shim, Frey Garabagi and K. Peter Pauls	<b>Molecular characterization of key genes for folic acid synthesis in common bean.</b>
P53	<u>Zeinab Yadegari</u> <sup>*</sup> and K. Peter Pauls	<b>Molecular mapping of genes involved in the phenylpropanoid pathway in common bean (<i>Phaseolus vulgaris</i> L.)</b>
P54	<u>Donna Yee</u> <sup>*</sup> , Jennifer N. Salt, and Daphne R. Goring	<b><i>Arabidopsis</i> Plant U-Box (PUB) E3 ubiquitin ligases have diverse regulatory roles during plant growth and development.</b>

## Abstracts

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# Keynote Abstracts

2008 CSPP-ERM

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Friday December 5th, 2008  
ROM, Level 1B, Signy & Cléopée Eaton Theatre

## **K1. Evolution and molecular development of bird pollinated flowers.**

Quentin Cronk

Department of Plant Science, University of British Columbia, UBC Botanical Garden and Centre for Plant Research, Vancouver, BC, V6T 1Z4, Canada

Evolutionary shifts to bird pollination from bee pollination have occurred many times in flowering plants. Bird pollinated flowers display a "syndrome" of common features which are responsible for (1) attraction of birds (2) deterrence of illegitimate flower visitors (particularly bees) (3) protection from vigorous foraging by birds, and (4) accurate placement of pollen on bird's bodies. This talk will examine shifts between bee and bird pollination in the context of flower evolution and developmental mechanisms, with special reference to the legume family. Recently, numerous genes have been identified as important in the development of the floral phenotype. Some of these are also implicated in shifts between bee-pollination and bird pollination, raising the prospect of a "molecular biology of bird pollination".

Saturday December 6th, 2008  
Earth Science Centre, Room 1050

**K2. Plant-bacteria warfare: Innate immunity & bacterial suppressors.**

Jen Sheen

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Plants possess innate immune systems to prevent infections and are effectively "nonhosts" for most potential pathogens. Our previous studies have revealed a fundamental role of convergent MAMP (microbe-associated molecular pattern) signaling in nonhost immunity. However, successful pathogens have evolved strategies to interfere with host immune systems. For example, the ubiquitous plant pathogen *Pseudomonas syringae* injects two sequence-distinct effectors, AvrPto and AvrPtoB, to intercept convergent innate immune responses stimulated by multiple MAMPs. We discover that AvrPto and AvrPtoB bind the Arabidopsis receptor-like kinase BAK1, a shared signaling partner of both the flagellin receptor FLS2 and the brassinosteroid receptor BRI1. This targeting interferes with ligand-dependent association of FLS2 with BAK1 during infection. It also impedes BAK1-dependent host immune responses to diverse other MAMPs and brassinosteroid (BR) signaling. Significantly, the structural basis of AvrPto-BAK1 interaction blocking MAMP signaling appears to be distinct from AvrPto-Pto association required for effector-triggered immunity. These findings uncover a unique strategy of bacterial pathogenesis where virulence effectors block signal transmission through a key common component of multiple MAMP-receptor complexes.

Saturday December 6th, 2008  
Earth Science Centre, Room 1050

**K3. Making starch – a simple glucose polymer requiring complex biochemistry.**

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Dept of Molecular and Cellular Biology, College of Biological Science, University of Guelph, N1G 2W1, Canada.

Starch is widely exploited in both food and non-food applications. In principle it is a simple, semi-crystalline polymer of glucose consisting of unbranched amylose, and more highly branched amylopectin. Its biosynthesis can largely be attributed to three classes of enzymes, the starch synthases (SS), starch branching enzymes (SBE), and debranching enzymes (DBE) for which multiple isozymes of each operate in the same sub-cellular location, the plastid. Considerable information on each enzyme has been derived from empirical genetic and biochemical studies, but we are far from being able to replicate starch synthesis *in vitro* or being able to make *in vivo* changes with predictable outcomes for defined functionality. Recent work has revealed mechanisms which underpin the biochemical coordination of the enzymes involved and contribute to the architecture of the starch made. In particular we have discovered that many of these enzymes form complexes, the assembly and disassembly of which is governed by protein phosphorylation. Further, genetic variation in individual steps also leads to alteration in the protein complexes which are formed. Understanding the assembly of starch biosynthetic enzymes into different protein complexes provides new insight into how biosynthesis is regulated *in vivo*, and offers novel opportunities for starch modification.

### **1A-1. Abiotic stress associated divergence in gene expression following an *Arabidopsis thaliana* whole-genome duplication.**

Gregory Downs<sup>1\*</sup> and Lewis Lukens<sup>1</sup>.

<sup>1</sup>Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Over 70% of all plant species are recent polyploids, and all plants likely have a polyploid ancestor. The signature of past polyploidization remains in the genome as collinear genes with significant identity within long chromosomal regions. Changes in gene regulation in response to stress may explain why some duplicate genes are maintained in the genome while others are lost. To test this hypothesis, we examine transcript levels of the ancient polyploid *Arabidopsis thaliana* subjected to abiotic stress (272 microarrays). Genes within a duplicate pair often differ in their response to stress. Across nine stress treatments in two tissue types, an average of 14% of the genes within duplicate pairs significantly differed in their stress response ( $p < 0.001$ ). Response by both genes is observed in only 2.7% of the duplicates on average. In addition, duplicate genes which differ in their response to stress are shared amongst relatively few stress conditions. Regulatory differences across gene pairs are not correlated with sequence divergence. Certain functional classes show greater divergence. Our results suggest that regulatory changes occur in specific gene classes and alter the genes' responses to abiotic stress. This greatly contributes to the maintenance of duplicate genes following a whole-genome duplication event.

### **1A-2. The *Populus* drought response is shaped by genotype and time of day.**

Olivia Wilkins<sup>1,2\*</sup>, Malcolm M. Campbell<sup>1,2</sup>

<sup>1</sup>Department of Cell & Systems Biology and <sup>2</sup>Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, Ontario, Canada, M5S 3B2

As exposure to episodic drought can impinge significantly on forest health and on the establishment of productive tree plantations, there is great interest in understanding the mechanisms of drought response in trees. Affymetrix GeneChip microarray analysis of two commercially important *Populus* clones (DN34 and NM6) was used to identify genes characterising the drought response for each genotype over a diurnal period. The clones differed significantly in both their physiological and transcriptomal responses to water withdrawal. The data derived from our studies provide insights into the variety of genetic mechanisms underpinning the *Populus* drought response, and provide candidates for future experiments aimed at understanding this response across this economically and ecologically important genus. This study emphasises the fact that it is not possible to draw simple, generalised conclusions about the drought response of the genus *Populus* on the basis of one species, nor on the basis of results collected at a single time point.

## 1A-3. *Populus* drought transcriptome: Intraspecific differences in transcriptome activity.

Erin T. Hamanishi<sup>1\*</sup>, Olivia Wilkins<sup>2</sup>, Sherosha Raj<sup>2</sup>, and Malcolm M. Campbell<sup>1,2,3</sup>

<sup>1</sup>Faculty of Forestry, <sup>2</sup>Department of Cell and Systems Biology, <sup>3</sup>Centre for the Analysis of Genome Evolution & Function, University of Toronto, Toronto, ON M5S 3B2

Throughout the Canadian landscape, trees of the genus are known for both their high ecological and economic value. However, drought is a major limitation to the growth and productivity of *Populus* trees. Responses to drought stress are highly variable among *Populus* trees. Our research aims to develop a mechanistic understanding of the interplay between genotype and environmental effects. Specifically, we addressed hypotheses related to how intra-specific differences in growth and productivity are influenced by water availability, and how these differences are underpinned by variation in transcriptome activity. The analysis of transcriptome activity of six different *Populus balsamifera* clones under both well-watered and water-deficit conditions were assessed using Affymetrix Poplar GeneChip technology. We show that transcriptome activity differs tremendously among *Populus balsamifera* clones in response to water-deficit conditions; however, certain genes do have similar patterns of expression across all individuals. This work highlights the fact that the response to water-withdrawal among individuals of the same species of poplar can be highly variable on a transcriptome-level. The data presented has implications in our understanding of the complexities of the drought response in *Populus*, and can provide candidate genes for future efforts at unravelling this complex process in such an important tree species.

## 1A-4. The Role of Alternative Oxidase during Low Temperature Growth in *Nicotiana tabacum*.

Steven(Jia) Wang<sup>\*</sup>, Sasan Amirsadeghi and Greg C. Vanlerberghe

Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON M1C 1A4

To study the potential role of mitochondrial alternative oxidase (AOX) in ROS balance and carbon metabolism at low temperature, we compared wild-type *Nicotiana tabacum* plants to transgenic plants with altered AOX expression levels. Before cold treatment, levels of lipid peroxidation (LP) were inversely proportional to levels of AOX, indicating AOX functions in dampening ROS generation. After the cold shift, AOX overexpressors displayed lower LP levels and unexpectedly stronger induction of key ROS-scavenging genes than WT. Strikingly, RI29 (an AOX silencer) showed a lower LP level accompanied by a larger increase in antioxidant defense compared with WT. However, RI9, a "weaker" AOX silencer, displayed a consistently higher LP level than WT and poor induction of ROS-scavenging genes. This contrasting result between RI9 and RI29 suggests that a threshold level of mROS regulated by AOX is necessary to provide a mitochondrial signal to activate antioxidant defense. Exposure to low temperature was accompanied by a large increase in the pool size of monosaccharides, which interestingly was proportional to levels of AOX after temperature shift. This result contradicts the idea that AOX may consume excess carbohydrates but suggests that more AOX may protect the function of chloroplasts from cold damage, facilitating sugar accumulation.

**1A-5. The role of the mitochondrion and alternative oxidase during *Pseudomonas syringae* infection of *Nicotiana tabacum* leaves.**

Marina Cvetkovska\* and Greg C. Vanlerberghe

Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON Canada M1C1A4

Mitochondria have an important role in cellular signalling, possibly through the production of reactive oxygen species (ROS) by the mitochondrial electron transport chain (ETC). Plant mitochondria have an alternative oxidase (AOX), which prevents over-reduction of the ETC during stress conditions, and thus modulates ROS production. We are investigating the potential role of AOX during biotic stress using two experimental systems: tobacco plants infected with a virulent pathogen and with two non-host pathogens (one which induces the appearance of HR-like lesions and a second one which induces defence responses without cell death); and tobacco suspension cells treated with concentrations of salicylic acid (SA) that induce necrotic death, PCD or defence mechanisms without PCD. We find that in both systems, AOX expression is induced very early during defence responses without the appearance of PCD, but not in the case of PCD appearance. In contrast, HIN1, HSR203J (HR-marker genes) and PAL (involved in SA synthesis) are upregulated early during the HR response. Based on these results, we hypothesize that the differences in gene expression reflect differences in SA accumulation during different plant-pathogen interactions, and we propose that AOX levels might control the scale and type of response (PCD, necrotic death or defence induction) of plants to pathogens.

**1A-6. Photoperiodic injury in tomato is linked to diel N metabolism and mediated by protein tyrosine nitration.**

L. Tian<sup>1\*</sup>, D' Silva<sup>2</sup>, B. J. Micallef<sup>1,2,3</sup>, and B. Grodzinski<sup>1,2,3</sup>

Department of Plant Agriculture, University of Guelph, Guelph, ON, Canada N1G 2W1

Photoperiodic injury (PI) of vegetative tissues occurs under either extended photoperiods or non-24 h light/dark cycles in tomato (*Solanum lycopersicum*). We subjected 'Basketvee' (BV) (PI-susceptible) and 'Micro-Tom' (MT) (PI-resistant) to 12 or 24 h light for 7 d. 24 h light led to both nitrite accumulation in leaves and a loss of circadian rhythms for stem elongation for 'BV' but not for 'MT'. To determine if a relationship exists between nitrite accumulation and circadian rhythms, we further tested diel activities and transcriptional levels of both nitrate reductase (NR) and nitrite reductase (NiR). For BV, the mRNA level of NR, a well known clock-regulated enzyme, stopped showing rhythmic fluctuations in the 24 h light treatment, while the 12h control maintained the rhythmic fluctuations. In contrast, the 'MT' NR-mRNA level showed circadian fluctuations under both a 12h and 24h photoperiod. We also tested the biochemical effect of nitrite accumulation in tomato, and found that continuous light caused tyrosine nitration of protein in leaf tissue only for the PI-susceptible cultivar. Our results suggest that PI in tomato occurs in part through nitrite toxicity mediated by tyrosine nitration of proteins, which is linked to a loss of circadian coordination between NR and NiR.



## **1A-7. Sorting of the *Carnation Italian ringspot virus* replication protein p36 to the mitochondrial outer membrane is mediated by an internal targeting signal and the TOM complex.**

Yeen Ting Hwang<sup>1\*</sup>, Andrew W. McCartney<sup>2</sup>, Satinder K. Gidda<sup>1</sup>, and Robert T. Mullen<sup>1</sup>

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*Tombusviruses* are positive-strand RNA plant viruses that, depending on the virus and/or host, cause peroxisomes or mitochondria in an infected cell to be transformed into multivesicular bodies (MVB), the sites of viral RNA replication. While the cytopathological features associated with MVB biogenesis are relatively well known, our understanding of the molecular mechanisms underlying the differential targeting of viral replication proteins to either peroxisomes or mitochondria, as well as the host-cell factors that facilitate these distinct sorting events, are limited. Here we report on the mitochondrial targeting of the 36-kDa RNA replication protein (p36) from the *Carnation Italian ringspot tombusvirus*. We show that the targeting information in p36 consists of its two moderately hydrophobic transmembrane domains and the positively-charged face of an amphipathic helix within the intervening loop sequence. We show also that p36 interacts with certain components of the translocon of the outer mitochondrial membrane (TOM complex), but not with the sorting and assembly (SAM) complex. Discussed is the significance of these results in terms of i) how viruses exploit specific host-cell protein sorting pathways to facilitate their replication and ii) how this information provides insight to the sorting of host-cell mitochondrial membrane proteins, a process that has been largely unexplored in plants.

## **1B-1. Novel Green to Red photo-convertible EosFP based probes for plants.**

Allison M. Sinclair, Mike Schenkel\*, Chris Trobacher, Daniel Johnstone, Devon Radford, Neeta Mathur, John Greenwood, J. Derek Bewley and Jaideep Mathur

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Since the cloning of the Green Fluorescent Protein and its successful expression in living cells fluorescent proteins have become integral components of the biologists' tool kit for understanding subcellular dynamics and interactions. A growing, frequently updated free web-resource provides comprehensive lists of subcellular-targeted fluorescent probes developed for plants (<http://www.illuminatedcell.com/>). Here we present recent advances in the field of FP-based research including the development of novel photo-convertible fluorescent proteins by our lab. These newly discovered proteins change their fluorescent properties radically in response to mild irradiation. Through local photo-activation or -deactivation of fluorescence, these probes permit specific highlighting of a subset of subcellular targets and thus raise the level of precision in live imaging. Our present focus is on EosFP, a photo-convertible protein derived from the scleractinian coral *Lobophyllia hemprichii*. EosFP fluorescence changes irreversibly from green (emission max. 516 nm) to red (emission max. 581 nm) upon illumination with a wavelength of approximately 400 nm. The targeting of EosFP to different subcellular organelles and cytoskeletal elements in living plant cells, techniques for efficient photo-conversion using epi-fluorescent and confocal laser scanning microscopes, methods for post-acquisition data processing

### **1B-2. Peroxule formation: An early intracellular response to specific forms of oxidative stress.**

Alison M. Sinclair\*, Neeta Mathur, Jaideep Mathur

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Organelle cross-talk and intracellular communication have long been of fascination to cell biologists. Plant cells are of particular interest, as rapid organelle pleomorphy including the formation of long extensions called stromules and matrixules from chloroplasts and mitochondria, respectively, are common features. Recently, extensions from peroxisomes have been recognized as "peroxules." Based on the hypothesis that peroxules may be involved in cellular detoxification of reactive oxygen species (ROS) *Arabidopsis* seedlings were subjected to various ROS-inducing conditions. Live imaging was used to determine that specific forms of ROS resulted in transient peroxule formation within seconds of exposure. However, exposure to UV light or hydrogen peroxide for more than 240 seconds invariably arrested peroxule formation and instead created elongated peroxisomes reaching lengths of up to 7  $\mu\text{m}$  (normal peroxisome diameter is 1-1.5  $\mu\text{m}$ ). Elongated peroxisomes subsequently broke up into smaller peroxisomes. This work creates a clear distinction between the early responses of peroxisomes aimed at local alleviation of ROS versus late responses that lead to increased peroxisomal numbers within a cell. The results are part of a proof of concept study that supports the creation of an 'Early Intracellular Response Profile of Plants' (EIRPP) using existing and easily available molecular cell biological resources.

### **1B-3. The N-terminal acidic domain of the Arabidopsis chloroplast preprotein receptors atToc132 and atToc159 are intrinsically unstructured.**

Lynn G.L. Richardson<sup>1\*</sup>, Masoud Jelokhani-Niaraki<sup>2</sup> and Matthew D. Smith<sup>3</sup>

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The Toc159 family of receptors in *Arabidopsis* recognize nuclear-encoded chloroplast proteins at the chloroplast outer envelope membrane. The Toc159 homologues in *Arabidopsis* (atToc159, atToc132, atToc120, atToc90) are able to distinguish between sub-classes of preproteins; however the mechanism of preprotein recognition is unclear. These receptors have a tripartite structure consisting of a central GTPase (G-) domain, a C-terminal membrane anchor (M-) domain, and an N-terminal acidic (A-) domain. Several properties of the A-domain are consistent with its classification as an intrinsically unstructured protein (IUP) domain including sensitivity to proteolysis, aberrant migration during SDS-PAGE, and an abundance of acidic amino acid residues. In this study, we use CD spectroscopy to show that the A-domain of atToc159 and atToc132 are unstructured at physiological pH. Furthermore, the A-domains show an increase in secondary structure with increasing temperature and decreasing pH, which are common characteristics of IUPs. Alpha helical content notably increases in the presence of trifluoroethanol, which suggests a high propensity for structure under suitable conditions. These results have important implications for future studies aimed at determining the function of the A-domain and may lend insight into details about the specificity of preprotein recognition and Toc complex assembly that remain unclear.

**1B-4. Protein import into chloroplasts in the single-cell  $C_4$  species *Bienertia sinuspersici*: Identification of translocons at the outer envelope membrane of chloroplasts.**

Terry S.C. Lung\*, and Simon D.X. Chuong

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Three Chenopodiaceae species have been shown to perform  $C_4$  photosynthesis in single chlorenchyma cells, contradicting the conventional dual-cell arrangement in terrestrial  $C_4$  plants. The single-cell  $C_4$  systems feature morphologically and biochemically distinct chloroplasts in two cytoplasmic compartments essential for the  $C_4$  functions. Although the development of these dimorphic chloroplasts has been studied at the ultrastructural and biochemical levels, little is known at the molecular level. To investigate how nuclear-encoded  $C_4$  enzymes are imported into the different chloroplasts within the same cell, we characterized the protein import machinery at the outer envelope membrane of chloroplasts in *B. sinuspersici*. We identified three translocon receptors (BsToc159, BsToc132, and BsToc34) which may play a role in precursor protein recognition. They share high homology with each other except that the two Toc159 isoforms (BsToc159 and BsToc132) contain additional cytosolic N-terminal domains which are absent in the Toc34 homologue (BsToc34). Despite the unknown functions of these N-terminal domains, the high variability (23% homology), acidity (predicted  $pI = 4.2\text{--}4.4$ ), and the presence of long stretches (36~79 residues) of tandem repeats implicate their putative role in selective binding of positively charged transit peptides. Future directions to understand the translocation machinery in relation to the differential chloroplast protein import in single-cell  $C_4$  system will be discussed.

**1B-5. The sorting of tail-anchored *Arabidopsis* OEP9, Toc33 and Toc34 is mediated by distinct targeting signals and the lipid composition of the plastid outer envelope.**

Preetinder K. Dhanoa<sup>1\*</sup>, Matthew P. Henderson<sup>2</sup>, Matthew D. Smith<sup>3</sup>, David W. Andrews<sup>2</sup> and Robert T. Mullen<sup>1</sup>.

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While the intracellular trafficking of mammalian and yeast tail-anchored (TA) proteins has been relatively well characterized, our understanding of TA protein biogenesis in plants is limited because only a few members of this group of integral membrane proteins have been identified. Here, we describe the results of a comprehensive series of *in vivo* and *in vitro* experiments aimed at characterizing the targeting and membrane insertion mechanisms for three putative TA chloroplast outer envelope proteins (OEPs) from *Arabidopsis*: OEP9, a novel 9-kDa OEP recently identified in a bioinformatics-based screen for candidate TA proteins in the deduced *Arabidopsis* proteome, and Toc33 and Toc34, two closely related OEPs that are known to function as substrate-specific GTPase receptors in chloroplast protein import, but have not been well studied in terms of their biogenesis. Overall, we show that all three proteins localize to the chloroplast outer envelope in a TA manner, as expected, but that OEP9 differs from Toc33 and Toc34 with regards to their molecular targeting signals and the specific lipid components required for their proper membrane insertion. These results, as well as those aimed at elucidating the role of cytosolic ankyrin-repeat proteins, AKR2A and AKR2B, in targeting these TA OEPs, will be discussed.

**1B-6. AtBXL1 encodes a bifunctional  $\beta$ -D-xylosidase/ $\alpha$ -L-arabinofuranosidase required for pectic arabinan modification in *Arabidopsis* mucilage secretory cells.**

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In *Arabidopsis*, the epidermal cells of the outer ovule integument differentiate through a complex process into specialized cells that produce mucilage between the primary cell wall and plasma membrane. Upon imbibition the mucilage expands rapidly, breaking through the primary cell wall and enveloping the seed. A mutation in *BXL1* causes a peculiar phenotype where mutants have patchy and delayed mucilage release compared with wild type seeds. These mutants appear to undergo normal mucilage production and mucilage secretory cell development. Cloning of *BXL1* by plasmid rescue revealed a T-DNA insertion in *AtBXL1*, a gene encoding a beta-xylosidase/arabinofuranosidase. Molecular complementation, and two independent Salk T-DNA knockout lines in the same locus producing a similar 'patchy' release phenotype, confirms the gene is involved in mucilage release. Expression analysis with real-time RT-PCR shows expression of *BXL1* in all tissues tested, including 7, and 10 day old seeds, consistent with its proposed role in mucilage release and cell wall modification. Chemical analysis suggests that *bx1* mutants have an increase in arabinose, specifically (1→5)-linked alpha-D-arabinofuranose in their seed coat mucilage when compared to wildtype. Immunofluorescence on whole and sectioned seeds suggests that the arabinan increase is primarily cell wall localized in the *bx1* mutant.

**1B-7. The role for *BLADE-ON-PETIOLE1* and *2* in conversion of shoots to flowers during the floral transition in *Arabidopsis*.**

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The transition from vegetative to reproductive development in *Arabidopsis* is controlled by both endogenous and environmental signals. *LEAFY* (*LFY*) and *APETALA1* (*AP1*) are key regulators of this transition and expression of these genes in organ primordia confers floral fate. We now show that *BLADE-ON-PETIOLE 1* and *2* function together with *LFY* and *AP1* to promote the transition from shoots to flowers during the floral transition. *bop1 bop2* double mutants show only subtle defects in inflorescence and floral architecture but in combination with *lfy* or *ap1* synergistic defects in floral-meristem fate and floral architecture are revealed. Our data indicated that BOP1/2 function redundantly with *LFY* to activate *AP1* in early floral induction and that all three activities converge to downregulate expression of flowering-time genes such as *AGAOUS-LIKE24* in floral primordia, an important early step in the conversion of shoots to flowers. Finally, BOP1/2 promotes determinacy in flowers by controlling spatial expression of the meristematic regulator *BREVIPEDICELLUS*. Taken together, our data indicate that BOP1/2 contribute to the conversion of shoots to flowers during the floral transition by 1) promoting floral-meristem identity, 2) repressing inflorescence identity, and 3) spatially regulating the expression of factors known to control inflorescence architecture and meristematic activity.

## 1C-1. Soybean stem residue for auto industry.

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Increased use of plant fibers in automotive parts is limited by their poorer performance in composites compared to glass and polypropylene fibres. Lignin and hemicellulose reduce the value of plant fibers because they degrade at lower temperatures than cellulose. The objectives of this research were to identify genes that contribute to soybean fibre performance in composites, map Quantitative Trait Loci (QTL) for fiber traits and develop fiber gene-specific markers. Gene-based PCR primers for 140 cell wall biosynthesis genes were tested with RG10 and OX948 genomic DNA. Polymorphic primers were screened in 169 recombinant inbred lines (RILs) of a RG10 x OX948 population and subsequently mapped. Monomorphic markers were sequenced to produce Single Nucleotide Polymorphism (SNP) markers. PAL2, 4CL2, NAD, laccase, peroxidase and COBL4 gene fragments were isolated and converted to easily scored markers. Fifty lines, selected on the basis of a height/lodging index, were evaluated under controlled and field conditions in 2008. Characterization of their fibre performance in composites is underway. The marker and performance data will be used to map fiber performance QTLs. The development of marker-based screening methods for desirable alleles of fiber performance genes will allow their rapid introgression into elite germplasm and agriculturally acceptable varieties.

## 1C-2. Overexpression of cytosolic glutamine synthetase in rice improves grain yield and nitrogen harvest index.

Liz K. Brauer<sup>1\*</sup>, Amanda Rochon<sup>1</sup>, Steven Rothstein<sup>2</sup>, Barry J. Shelp<sup>1</sup>

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Plants use approximately 50% of the nitrogen (N) applied as fertilizer, with the remaining N often contributing to the pollution of water systems and the atmosphere. One strategy for reducing agricultural pollution caused by fertilizer-N is to increase the N use efficiency (NUE) of the plant. Recent literature suggests that enhancing the activity of glutamine synthetase (GS), the enzyme responsible for primary assimilation of N into glutamine, can influence N remobilization and NUE. Three independent cytosolic GS overexpressor lines of rice were generated by *Agrobacterium*-mediated transformation and identified using quantitative real-time RT-PCR and activity assays (1.7-2.8 times the azygous control). Preliminary physiological and biochemical characterization of the transgenic lines under limiting N revealed that: 1) the amino acid concentration in the flag leaf was not significantly different among lines or N treatments; 2) the biomass harvest index of two of the three overexpressor lines was significantly increased (22-27%); and 3) the nitrogen harvest index was significantly increased by 24% in the only tested line. Both biomass and nitrogen harvest indices were positively correlated to GS activity, and shoot biomass and N content were unchanged, suggesting that the positive influence of GS overexpression was due to altered N partitioning.

## Contributed Seminar Abstracts

2008 CSPP-ERM

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### 1C-3. Laboratory Assays Confirm Native uses of sweet fern (*Comptonia peregrina*) for its medicinal properties.

Matthew Edward<sup>1\*</sup>, Breanne Duquette<sup>1</sup>, Bill Dew<sup>1</sup>, Philippe Babady-Bila<sup>1</sup>, Breanne Copeland<sup>1</sup>, Tony Parkes<sup>1</sup> and Ewa Cholewa<sup>1</sup>

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Sweet fern (*Comptonia peregrina*) grows abundantly in Northern Ontario. Native people have used Sweet fern as an anti-inflammatory, to relieve congestion, and to treat skin ailments. In this study, crude water, butanol, ethyl acetate, dichloromethane, hexane and essential oil extracts of Sweet fern were investigated for their antioxidant and antibacterial activities. Antioxidant capacity was determined using the oxygen radical absorbing capacity (ORAC<sub>FL</sub>) method and the diphenylpicrylhydrazyl (DPPH) method. The antioxidant capacity of the extracts ranged from 25.097 ± 0.104% to 53.952 ± 0.856% µM TROLOX equivalents per 0.01 g/L of extract, with crude water having the lowest antioxidant activity and ethyl acetate having the highest. A method using *Drosophila melanogaster* mutants sensitive to oxidative stress is being developed to evaluate *in vivo* anti-oxidant capacity of Sweet fern. The disc diffusion assays revealed that both the ethyl acetate and butanol extracts inhibited growth of *Bacillus subtilis* (gram positive) and *Alcaligenes faecalis* (gram negative) indicating that antibacterial properties of sweet fern is not dependent on gram status of bacteria. In addition, extracts strongly inhibited *Staphylococcus aureus*, a bacterial strain which causes skin infections, confirming the validity of Native uses of sweet fern for skin disorders.

### 1C-4. Pot size matters.

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Studies involving pot-grown plants represent an underpinning of comparative plant physiology. However, design parameters such as the size of the experimental microcosm (e.g. pot size) may confound the interpretation of results regardless of the intended experimental conditioning. Specific to the study of mycorrhiza – a mutualistic association between plant roots and soil fungi – we believe that the pot size and fungal inoculum distribution may inadvertently affect plant growth and symbiotic interaction. From two factorial greenhouse experiments involving a 'dwarf' sunflower cultivar and an arbuscular mycorrhizal (AM) fungus, we report that large-potted plants developed a greater overall biomass and AM root colonization than small-potted ones due to the larger 'rootable' volume. Meanwhile, plants grown in a high density layered inoculum substrate showed a more advanced level of root colonization than those grown in a dispersed inoculum substrate, likely due to a higher probability of plant-fungus interactions. In view of facilitating the comparison of findings among pot-growth studies, we provide here practical implications for reducing result biases attributed to experimental design factors. As for pot size, it would seem that "bigger is better".



**1C-5. Flow cytometric measurements of oxidative stress in freshly isolated algal symbionts as a function of temperature and iron availability to coral colonies.**

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The photophysiology of coral-algae symbioses is critical to the survival of global coral reefs. We used flow cytometry and intracellular fluorescent probes to assess the photophysiology of freshly isolated zooxanthellae (FIZ) from the coral *Stylophora pistillata*. Intracellular reactive oxygen species (ROS) were measured using the fluorescent dyes, dihydroethidium and CMH<sub>2</sub>DCFDA, and cell membrane integrity was assessed using SYTOX, a cell wall impermeable dye. We proposed that greater photophysiological stress would be observed in FIZ isolated from *S. pistillata* colonies under high temperature (31°C) relative to control colonies (27°C). We proposed stress would be greater at higher temperatures when iron availability was low; conditions created in our cultures by adding the strong iron chelator desferrioxamine B. Our results showed that FIZ contained a significantly lower level of ROS (superoxide radical) and maintained a higher than predicted level of cell membrane integrity under low iron, high temperature conditions—consistent with an induced photo-protective mechanism. These findings are consistent with increases in photoprotective pigment in these FIZ, implying that diminished iron availability renders corals more susceptible to decreased photosynthetic efficiencies under bleaching conditions.

**1C-6. Investigation of the factors impacting mycorrhizal density in natural populations of *Fragaria virginiana*.**

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Arbuscular mycorrhizae (AM) represent a common symbiotic association between plant roots and soil fungi. It is known that a variety of factors can impact the density of fungal association. In the current study, the VAM density of wild strawberry plants (*Fragaria virginiana*) was assessed across different environments and through the reproductive cycle. The plants were wild populations found at five locations around the Pymatuning State Park (Jamestown, PA). Plants were sampled from mid-May to mid-July, spanning the flowering, fruiting and post-fruiting portions of *F. virginiana* reproduction. Plant sex, *F. virginiana* is a gynodioecious species, was also factored into the evaluation. To directly assess the fungal density in the mycorrhizal association, second- and third-order roots were cleared and stained with Trypan blue. The quantity of AM structures was then scored using a microscope. Additionally, soil from around the root system was screened to determine the spore density, which served as a rough indicator of fungal reproduction. While initial studies found no difference, more intense sampling revealed that both reproduction and environment had slight but significant impacts on mycorrhizal density. This was among the first observed impacts of *F. virginiana* reproduction on measureable plant parameters.

**1C-7. High stability ferric chelates result in decreased iron uptake by the green alga *Chlorella kessleri* due to decreased ferric reductase activity and chelation of ferrous iron.**

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Cells of the green alga *Chlorella kessleri* use a reductive mechanism for iron acquisition. Iron-limited cells acquired iron more rapidly from a chelator with a lower stability constant (HEDTA) for Fe<sup>3+</sup> than from a chelator with a higher stability constant (HBED). Furthermore, iron uptake rates decreased with increasing chelator concentrations at constant iron concentration. The negative effects of elevated HBED levels on iron uptake could be partly alleviated by the addition of Ga<sup>3+</sup>; this suggests that iron-free chelator has a negative effect on iron acquisition by competing with the ferrous transport system. Furthermore, ferric reductase activity progressively decreased with increasing concentrations of both chelators (in the iron-free form); this effect was not alleviated by Ga<sup>3+</sup> addition and was apparently caused by the direct inhibition of the reductase. Overall, we conclude that chelators with high stability constants for Fe<sup>3+</sup> decrease iron acquisition rates by Strategy I organisms via three separate mechanisms: 1) chelation of the Fe<sup>2+</sup> produced by ferric reductase activity (partially alleviated by Ga<sup>3+</sup>), in competition with the Fe<sup>2+</sup> transport system, 2) decreased ferric reductase activity compared to lower stability ferric chelates, and 3) progressive inhibition of ferric reductase activity at increasing concentrations of iron-free chelator (not alleviated by Ga<sup>3+</sup>).

**2A-1. CBB Resistance in *Phaseolus vulgaris*: Towards the Identification of a Resistance Gene.**

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Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* is a ubiquitous disease in dry bean (*Phaseolus vulgaris*) that results in leaf, pod and seed lesions and significant reductions in yield and seed quality. Recently, a CBB-resistant cultivar, OAC-Rex was developed from a cross between *P. vulgaris* and a CBB-resistant accession of *Phaseolus acutifolius*. Another CBB-resistant line, HR67, was produced from a separate cross between *P. vulgaris* and *P. acutifolius*. OAC-Rex represents the first CBB resistant cultivar released in North America, however the genes responsible for this resistance not yet been identified. To aid in the identification of CBB-resistance genes, binary-bacterial artificial chromosome (BIBAC) libraries were created from OAC-Rex and HR67. The libraries were screened with CBB resistance-associated molecular markers identified by previous studies, and the identified clones were analyzed and assembled into contigs. The fragments at the extreme ends of the contigs were sequenced; from which new probes were derived to re-probe the libraries and expand the coverage of the contig. Inoculation of *Arabidopsis thaliana* leaves with *X. axonopodis* resulted in a similar symptomology to that observed in *P. vulgaris*. Bacterial growth assays have also indicated that within 96hr post infection, there is a 1000-fold increase in bacterial concentration in inoculated leaves, suggesting that *Arabidopsis* is a potential host for the pathogen. The unique clones will be transformed into *A. thaliana* and screened with *X. axonopodis* to identify clones with potential CBB resistance genes.

**2A-2. Investigation of DIR1 movement during long distance signalling in systemic acquired resistance in *Arabidopsis*.**

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Systemic Acquired Resistance (SAR) is an induced resistance mechanism in which certain localized pathogen infections lead to broad resistance against future attacks in distant tissues. Resistance is transmitted from induced tissues by long distance signals that are perceived in systemic tissue eliciting defence to normally virulent pathogens. Our previous research indicates that DIR1 may function as a long distance signal during SAR. *Agrobacterium* transient transformation was used to study DIR1 movement in the *dir1-1* SAR-defective mutant. Lower leaves of *dir1-1* were infiltrated with *Agrobacterium* expressing DIR1:YFP, followed by inoculation with SAR-inducing *Pseudomonas syringae* pv *tomato* (*Pst* *avrRpt2*). Distant leaves were challenged with virulent *Pst* and bacterial levels were determined. The SAR defect was rescued in *dir1-1* plants expressing DIR1:YFP only if SAR was induced. Petiole exudates were collected from these induced leaves and DIR1 was present suggesting that DIR1 is capable of movement during SAR. A similar rescue response was observed when *dir1-1* was infiltrated with petiole exudates from SAR induced cucumbers that contain a DIR1-sized product. This suggests that cucumber exudates contain a DIR-like protein and that both species utilize similar long distance SAR signaling mechanisms.

**2A-3. A novel *Arabidopsis* SAR mutant, *pac2*, shows enhanced hypersensitive cell death in response to pathogen infection.**

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Systemic acquired resistance (SAR) is a critical component of plant immunity that occurs in uninfected systemic tissue following infection by an avirulent pathogen or exogenous application of synthetic SAR activators. SAR is characterized as long-lasting and broad-spectrum where the plant acquires immunity to subsequent infections by a wide variety of pathogens. Although recent research has elucidated some components in the signal pathway for SAR, a complete molecular mechanism is still unknown. Current research has suggested the involvement of cyclic adenosine monophosphate (cAMP) in plant defense responses; furthermore, cAMP has been identified in several plant species. However, clear evidence of its biosynthetic protein, adenylylate cyclase (AC), is still lacking in plants. Therefore, a bioinformatics analysis was conducted and three genes were identified that possess an AC domain. Homozygous T-DNA insertion knockout mutants were generated for all three genes and a characterization of these knockout mutants was performed. Primary infection of *AtPAC2* knockout mutants with *Hyaloperonospora parasitica* revealed enhanced hypersensitive cell death in both infected and uninfected leaves. Upon secondary infection, these mutants exhibited enhanced SAR, displaying virtually no hyphal growth. These results indicate this putative AC gene may act as a negative regulator in SAR signalling.

## 2A-4. Investigation of abiotic stress responses in the pathogen resistant mutant *cpr22*.

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The *cpr22* mutant is an *Arabidopsis* lesion mimic mutant exhibiting spontaneous cell death, constitutively expresses the pathogenesis related genes *PR-1*, *PR-2* and *PR-5*, accumulates elevated levels of salicylic acid and displays an enhanced resistance to *Hyaloperonospora parasitica* Emco5. *cpr22* has a deletion in a cluster of cyclic nucleotide-gated ion channel genes, resulting in an in-frame chimeric fusion of the genes *AtCNGC11* and *AtCNGC12*. Interestingly, when *cpr22* plants were grown in high relative humidity, all the above *cpr22*-related phenotypes were suppressed. To investigate this environmental sensitivity further, genome wide transcriptome analyses were conducted to assess changes in gene expression after a 24 hour shift from 95 to 65 percent relative humidity. Abscissic acid (ABA) related gene expression was altered in *cpr22* after humidity shift. Hormone quantification also revealed that the amount of ABA accumulation in *cpr22* is two fold greater than in wildtype after the humidity shift. Furthermore, we have shown that *cpr22* has an impaired response to ABA induced dormancy in germination assays compared to wildtype, and has an accelerated loss of fresh weight during dehydration compared to wildtype. Taken together, these results suggest that *cpr22* has an attenuated response to abiotic stress, possibly due to altered ABA related signaling.

## 2A-5. The *Pseudomonas syringae* Type III Effector HopF2<sub>Pto</sub> DC3000 suppresses PAMP- and Effector-Triggered Plant Immune Responses.

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*Pseudomonas syringae* employs a type III secretion system (TTSS) to translocate type III secretion effector (TTSE) proteins into plant cells. Upon entry into the cytosol, numerous TTSEs are thought to manipulate components of the host molecular machinery in order to promote bacterial infection. Although many *P. syringae* TTSEs remain uncharacterized, some have demonstrated the ability to suppress the two major levels of plant innate immunity; PAMP-Triggered Immunity (PTI) and Effector-Triggered Immunity (ETI). Here we demonstrate that the TTSE HopF2<sub>Pto</sub> promotes *P. syringae* growth in *Arabidopsis thaliana*. Expression of HopF2<sub>Pto</sub> in *Arabidopsis* enhances growth of TTSS-system deficient *P. syringae* pv. *tomato* (Pto) DC3000  $\Delta$ hrcC mutant and suppresses flg22-elicited callose deposition associated with defence responses demonstrating that HopF2<sub>Pto</sub> can suppress PTI. We also demonstrate that HopF2<sub>Pto</sub> can effectively suppress ETI elicited by AvrRpt2 and to a lesser extent AvrRpm1- and AvrB- but not HopZ1a-mediated ETI. Further, AvrRpt2-mediated RIN4 degradation is compromised in HopF2<sub>Pto</sub>-expressing plants. Our results demonstrate that HopF2<sub>Pto</sub> targets important components of plant innate immunity in *Arabidopsis*, thereby suppressing defence responses and promoting pathogen virulence.

**2A-6. Chemical genomic investigation of the *Arabidopsis thaliana*-*Pseudomonas syringae* pathosystem.**

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The interactions between plants and their pathogens involve a complex array of molecular events that remain to be fully elucidated. In order to investigate the *Pseudomonas syringae*-*Arabidopsis* interaction in a high-throughput manner, we have devised a liquid assay using standard 96-well plates. We demonstrate that *Arabidopsis* seedlings incubated with *P. syringae* in liquid culture display a macroscopically visible "bleaching" symptom within five days of inoculation. Bleaching is associated with a loss of chlorophyll from cotyledonary tissues and is correlated with bacterial virulence. Based on this symptom, we initiated a screen intended to identify small molecules that reduce the susceptibility of *Arabidopsis* to infection by *P. syringae*. A screen of chemicals active on *Arabidopsis* revealed a family of sulfanilamide compounds that afford protection against the bleaching symptom. The most active compound, sulfamethoxazole (Smex), also reduced *in planta* bacterial growth when applied to soil-grown plants. A forward genetic screen is currently underway to determine the genetic basis for Smex-mediated resistance in *Arabidopsis*.

**2A-7. HopZ1a: a *Pseudomonas syringae* type III effector protein recognized by the RAZ1 resistance protein of *Arabidopsis thaliana*.**

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The plant pathogen *Pseudomonas syringae* uses a type III secretion system to secrete and translocate effector proteins directly into plant cells. A number of *P. syringae* type III effectors have been demonstrated to suppress host defense signaling and promote pathogen growth. On the other hand, recognition of specific type III effectors by plant resistance (R) proteins can induce an effective resistance response that is often accompanied by localized cell death termed the hypersensitive response. The YopJ / HopZ family of type III effectors is a common and widely distributed class found in both animal and plant pathogenic bacteria. The *P. syringae* HopZ family includes the closely related allelic variants HopZ1a, HopZ1b and HopZ1c, as well as HopZ2 and HopZ3, which were brought into this species via horizontal gene transfer. Ma et al (2006) showed that HopZ diversification was driven by the host defense response. We demonstrated that the virulence and defense induction phenotypes are strongly HopZ allele-specific and that the HopZ1a-induced defense response is independent of known R genes (Lewis et al., 2008). Our goal is to identify host targets of the five HopZ members, as well as the R protein that recognizes HopZ1a; and to determine how evolutionary adaptation has molded these interactions. This work will address how differences in host target specificity within one family of type III effectors contribute to the host specificity in this important pathogen.

## Contributed Seminar Abstracts

2008 CSPP-ERM

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### **2A-8. Dissection of plant resistance to pest using a genomic approach: *Arabidopsis*-Two Spotted Spider Mite *Tetranychus urticae*, novel model for plant-herbivore interactions.**

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In response to herbivore attack, plants have evolved a variety of mechanisms to deter herbivore feeding, which prevent the herbivores from jeopardizing the plant's health, reproduction, and ultimately survival. Understanding the fundamental mechanisms of plant resistance to pest represent the basis for breeding of pest-resistant crops. Our group is coordinating the *T. urticae* whole genome sequencing project that will provide genomic tools to dissect mite response to feeding on various hosts. In addition, we characterized the resistance among natural *Arabidopsis* accessions to spider mite damage and isolated *Arabidopsis* accessions resistant and susceptible to *T. urticae*. We profiled the transcriptome of naturally resistant and susceptible *Arabidopsis* accessions upon spider mite feeding using *Arabidopsis* microarray. We isolated genes induced by spider mite feeding in susceptible and resistant *Arabidopsis* ecotypes including potential candidate genes for plant resistance to spider mites. Dissecting plant-herbivore interactions on the genomic level will open new opportunities for control against major pest in agriculture.

### **2B-1. Ethylene receptors: A wealth of knowledge beyond the hypocotyl model.**

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Despite its simplicity in synthesis and structure, the hormone ethylene affects a multitude of plant developmental processes in a complex, and sometimes contradictory, manner. Perhaps the single most studied effect of ethylene has been on hypocotyl growth and mediation of the 'triple response'. These studies have formed our current understanding of ethylene signal transduction, but there are numerous other pathways that are much less studied that may also help our understanding of ethylene's role in plant development. We have studied ethylene's role in cellular development, defence and seed dormancy in *Arabidopsis* and found that the ethylene receptors do not have equal roles in development. In trichomes, ETR2 plays a role in cytoskeleton organisation. We show that ETR1 and EIN4 have opposing functions in mediating cell death and that each receptor has a different role in activating defence pathways. In seeds we demonstrate that ERS1 and ETR1 play opposing roles in seed dormancy through affecting sensitivity to the GA and ABA pathways. Together these results have led us to create a new model of how ethylene signalling works in different cellular processes – a model of non-redundant actions between the receptors that is key in explaining ethylene's role in plant development.



**2B-2. Screening a potato activation tagged mutant population identifies important developmental regulators involved in flowering and tuber development.**

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The potato is the fourth most consumed food crop in the world, and carries significant economic weight in Canada. In an effort to better understand the genes that are vital to potato growth and development, the Canadian Potato Genome Project (CPGP) generated more than 8500 activation-tagged potato lines which manifest as dominant, gain-of-function phenotypes. A preliminary greenhouse screen of 677 lines was performed and several prominent mutants were identified with morphological anomalies in the leaves, stems, flowers or tubers. One interesting mutant, named Twiggy Lafleur, exhibited increased stem branching and flowered earlier as well as longer compared to wild-type. A modified TAIL-based PCR strategy revealed the enhancer T-DNA inserted near a MADS-box transcription factor, that when over-expressed in tobacco and potato, shows a phenotype similar to that observed in the activation-tagged line. A second mutant, named Chocolate, displays a striking tuber phenotype characterized by a thick, dark-brown coloured skin. The enhancers appear to have inserted close to a putative MYB transcription factor that is up-regulated up to eighteen times in the Chocolate skin. Taken together, these results show that the activation-tagged population holds great promise for the identification of important developmental regulators in the potato genome.

**2B-3. Regulatory roles of plant microRNAs and small Interfering RNAs in *Arabidopsis thaliana*.**

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Two classes of non-coding small RNAs, microRNAs (miRNAs) and small interfering RNAs (siRNAs), guide several biological processes including heterochromatin assembly, mRNA cleavage and translational repression in a sequence-specific manner. To discover the regulatory functions of miRNAs and siRNAs, we have collected and analysed *Arabidopsis thaliana* transcriptome data from siRNA biogenesis loss-of-function mutants. Our results show that siRNA biogenesis mutants affect the transcript abundance of a small number of genes, suggesting that siRNAs are relatively unimportant for gene regulation. In contrast, miRNA biogenesis mutants affect the transcript abundance of a large number of genes. Some of the genes up-regulated in the miRNA mutants are known miRNA targets, and the analyses of GOslim molecular function classes of the miRNA up-regulated genes show that transcription factors occurred twice as frequently within the up-regulated group as compared to the non-up-regulated group. Genes up- and down-regulated by miRNAs within the inflorescence tend to be highly expressed in the inflorescence. These results are consistent with observations that miRNAs degrade expressed transcripts in a cell-specific fashion within a tissue. Interestingly, genes up-regulated in miRNA biogenesis mutants within the inflorescence are highly expressed in leaves, and genes down-regulated in miRNA biogenesis mutants are expressed at low levels in leaves. Thus, loss of miRNAs causes the inflorescence transcriptome to more resemble the leaf transcriptome. Our results will contribute to a functional understanding of miRNAs and siRNAs in plant development.

### **2B-4. AtMBD9 modulates *Arabidopsis* development through the dual epigenetic pathways of DNA methylation and histone acetylation.**

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Mutations within the *Arabidopsis* METHYL-CPG BINDING DOMAIN 9 gene (*AtMBD9*) cause pleiotropic phenotypes including early flowering and multiple lateral branches. Early flowering was previously attributed to the repression of Flowering Loci C (*FLC*) due to a reduction in histone acetylation. However, the reasons for other phenotypic variations remained obscure. Recent studies suggest an important functional correlation between DNA methylation and histone modifications. By investigating this relationship, we have found that the global genomic DNA of *atmbd-9* was over-methylated, including the *FLC* gene region. Recombinant AtMBD9 does not have an *in vitro* detectable DNA demethylation activity, but rather has histone acetylation activity. Ectopic overexpression of *AtMBD9* and transient DNA demethylation promotes flowering and causes partial recovery of the normal branching phenotype. Co-immunoprecipitation assays suggest that AtMBD9 interacts *in vivo* with some regions of the *FLC* gene and binds to histone 4 (H4). In accordance with this result, AtMBD9 itself was found to be localized in the nucleus and expressed in the flower and axillary buds. Together, these results suggest that AtMBD9 controls flowering time and axillary branching by modulating gene expression through DNA methylation and histone acetylation. This reveals another component of the epigenetic mechanism controlling gene expression.

### **2B-5. Analysis of *HUA2 LIKE* (HULK) gene family in *Arabidopsis thaliana*.**

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*HUA2* gene is a putative pre-mRNA processing factor involved in the regulation of flowering time and floral development. There are three other *HUA2 LIKE* (*HULK*) genes in *Arabidopsis* genome, named *HUA2 LIKE1* (*HULK1*), *HUA2 LIKE2* (*HULK2*), and *HUA2 LIKE3* (*HULK3*). The *HULK* gene family of proteins range from 1347 to 1445 amino acids, and they share 19 to 53% amino acid identities over their entire length. In addition, these *HULK* proteins contain the same conserved domains identified in *HUA2*: PWWP domain, RPR (pre-mRNA processing domain), proline-rich region containing PPLP repeats (no PPLP repeat in *HULK3*), and nuclear localization signals. *HULK* proteins have not been identified in animal species, indicating that this family of proteins may play roles unique to the plant development. Developmental and tissue specific expression patterns of members of *HULK* gene family are largely overlapping, suggesting possible functional redundancy. T-DNA insertional lines of *HULK1*, *HULK2* and *HULK3* did not show any obvious phenotypes. However, double mutant combinations showed distinct phenotypes: early and late flowering (depending on the combination), early sterile flowers, reduced fertility and embryo defects. Some of the triple mutant combinations are not viable. These data suggest that *HULK* gene family members may have redundant roles in flowering time and other aspects of plant development.

**2B-6. Heat stress induces autophagic programmed cell death of microspore mother cells in *Oryza sativa* (var. japonica).**

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The world's rice crop is currently exposed to temperature thresholds that drastically reduce grain production. We investigated the impact of 32°C and 36°C on pollen development and grain yield in rice. Results indicate that 32°C and 36°C trigger severe yield reductions by different processes. Anther indehiscence is the cause of failed grain production at 32°C. In contrast, at 36°C, reproductive failure is caused by abortion of microspore mother cells (MMCs). The early stages of MMC abortion are typified by a cytoplasm which contains autophagosomes and a nucleus with nuclear pore clusters, a dilated and cleaved nuclear envelope, and condensed chromatin which is immunopositive for ssDNA following a DNA denaturation assay. The late stages of abortion are characterized by degradation of the MMC cell wall and all protoplasmic components. These cellular processes, which are initiated in the absence of abnormalities in other anther tissues, are indicative of autophagic cell death and nuclear apoptosis. Although salt stress has been reported to cause autophagic cell death within female gametophytes, the observation that high temperatures induce autophagic cell death of MMCs is novel. Results provide crucial stage specific information for identification of molecular processes involved in the failure of grain production at high temperatures.

**2B-7. Developmental Programmed Cell Death (PCD) in Lace Plant (*Aponogeton madagascariensis*).**

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Perforation formation in the lace plant (*Aponogeton madagascariensis*) leaf is a fascinating example of developmental programmed cell death (PCD) in plants. In this system, discrete populations of cells enclosed by longitudinal and transverse veins (centre cells) undergo PCD, whereas cells within 5 cell layers of the veins (control cells) do not. The morphology of its leaf (thin cuticle and only 4 cell layers thick) and the predictability of cell death (both in timing and location) make the lace plant an excellent model for *in vivo* microscopic investigations. In dying cells, characteristic changes involving chloroplasts, nuclei, and actin microfilaments have been observed. Interestingly, dividing, dumbbell-shaped chloroplasts persisted until the late stages of PCD and chloroplasts formed a ring around the nucleus. Initial results using phalloidin to stain tissue mounts indicate changes in actin microfilaments as PCD progresses. In addition, preliminary results of transformation have indicated the possibility of transformation of lace plant tissues, thus enabling further characterization of the above PCD events *in vivo*. This unique plant is an excellent model for studying developmental PCD in plants, yet much work remains to better understand the cellular changes that occur during PCD.

## **2B-8. Network models in auxin signal transduction and vascular tissue patterning.**

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Feedback-regulated flows of auxin have been implicated in a stunning diversity of patterning processes in plants and have become subject to mathematical modeling. In these feedbacks, Auxin Response Factors (ARFs) have critical, partially overlapping functions in controlling the expression of AtPIN auxin-efflux associated proteins. They can be used as genetic tools to locally manipulate auxin signal transduction and auxin transport. In a redundancy matrix of an ARF subclade we have discovered a unique contribution of ARF5/MP in mediating PIN gene expression and have begun determining direct targets of ARF5/MP to identify key-regulators in various patterning decisions. We have further used genetic and experimental interference tools to dissect the formation of the *Arabidopsis* leaf venation pattern. AtPIN1 is expressed in domains that become restricted toward sites of procambium formation. Auxin flow polarities in emerging veins indicate auxin drainage toward pre-existing veins, but veins display split polarities as they become connected at both ends. Subcellular AtPIN1 polarity indicates that auxin is also directed to distinct "convergence points" in the epidermis, from where it defines the positions of major veins. Our results suggest that epidermal "convergence points" are part of a more general developmental module defining not only the positions of major leaf veins, but also the positioning of lateral shoot organs. These multiple readouts should facilitate experimental tests of mathematical models in leaves as well as meristems.

## **2C-1. Nuclear targeting of methyl recycling enzymes is mediated by a specific protein-protein interaction.**

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Both S-adenosyl-L-homocysteine hydrolase (SAHH; EC 3.3.1.1) and adenosine kinase (ADK; EC 2.7.1.20) are essential to sustain the hundreds of the methyltransferase (MT) activities required for plant growth. Both enzymes have generally been regarded as cytosolic enzymes despite the fact that SAM-dependent methylation reactions occur in all cellular compartments to maintain the MT activities throughout the cell. There are, however, no other reports of SAHH movement and no information on ADK being targeted to other compartments. To investigate this, we tested GFP fusion proteins of ADK or SAHH in plant cells and found that both GFP fusions were localized to the cytosol as previously reported by other research groups; in addition, they were present in the nucleus, even though the primary amino acid sequence of neither protein contains a detectable nuclear localization signal. To verify if the multiple location of both enzymes is mediated by the interactions or trafficking of other proteins, several protein-protein interaction assays were performed including bimolecular fluorescence complementation (BiFC), yeast 2-hybrid, StreptII-tag purification, and pull-down assays. The results suggest a possible interaction between ADK, SAHH, and other proteins. These interactions may be essential for the nucleo-cytoplasmic trafficking of ADK and SAHH that may contribute in the transmethylation cycle.

## 2C-2. 5'-methylthioadenosine nucleosidase-deficient plants exhibit developmental abnormalities.

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S-adenosylmethionine is the key methyl donor in plants and is also a precursor for polyamine (PA), nicotianamine (NA) and ethylene biosynthesis. These three biosynthetic pathways generate methylthioadenosine (MTA) as a by-product, and re-cycle it via the Yang cycle. In this cycle 5'-methylthioadenosine nucleosidase (MTN) irreversibly hydrolyses MTA to methylthioribose which is converted back to Met. The *Arabidopsis thaliana* genome has two genes encoding MTN: AtMTN1 and AtMTN2. Mutants of both isoforms (*mtn1-1* and *mtn2-1*) appear phenotypically normal. The double mutants (*mtn1-1mtn2-1*) show pleiotropic effects including interveinal chlorosis, thicker veins, reduced polar auxin transport and abnormal flower production. Although *mtn1-1* and *mtn1-1mtn2-1* mutants show increased MTA accumulation relative to the wild type, it is not obvious why the loss of MTN activity has this effect. The thicker veins were previously reported for a mutant of polyamine biosynthesis. Thus accumulation of MTA in MTN-deficient mutants may inhibit specific enzyme activities of PA biosynthetic pathway. Moreover, the interveinal chlorosis and abnormal flowers observed in these mutants are similar to previously reported NA-deficient plants. These results of genetic and physiological analyses along with metabolic profiling are underway to elucidate the impact of MTN deficiency on plant development.

## 2C-3. Adenosine kinase deficiency alters cell proliferation and cytokinin profiles in *Arabidopsis thaliana*.

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Adenosine kinase (ADK) catalyzes the phosphorylation of adenosine to adenosine monophosphate. By contributing to purine salvage, ADK plays an important role in maintaining energy levels, methylation and has been implicated as a means of inactivating cytokinin phytohormones. In order to fully elucidate the role of this enzyme, ADK-deficient lines were sought. As complete knockouts of ADK result in embryo lethality, two silencing lines were created by over-expressing a full length ADK cDNA in the sense orientation (sADK) and using artificial microRNAs (amiADK). Homozygous transgenic lines displayed a range in silencing with the most affected sADK individuals retaining 7-9% ADK activity and the amiADK having 5-6% residual activity. Common phenotypes shared by the two lines included small, wavy leaves, reduced apical dominance and enlarged meristems. Closer examination of the leaves revealed altered cell proliferation, in particular an increased number of cells and callus formation. As cell proliferation is tightly controlled by hormone signalling, cytokinin levels were measured using LC/MS/MS. Results show cytokinin nucleotide levels to be significantly increased in both sADK and amiADK lines. Future study will focus on the effect of this increased cytokinin activity in relation to other hormones and plant development.

### **2C-4. Investigating the role of APT1 Isoforms in purine salvage and subcellular localization.**

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APT1 is a constitutively expressed purine metabolic enzyme that catalyzes the salvage of adenine to adenosine monophosphate in *Arabidopsis thaliana*. Previous research identified one transcript arising from the APT1 locus; where deficiency in APT1 leads to aborted pollen development. However, current EST data has uncovered a second larger transcript that differs by an additional 5' terminal exon. Using semi-quantitative RT-PCR we found an equal transcript abundance of both transcripts in leaf and floral organs. However, western blotting reveals an abundance of the shorter protein product but undetectable levels of the larger protein product in both organs. Interestingly, fusion of the novel 5' terminal exon to GFP, under the control of the native APT1 promoter, provides evidence that translation can initiate at this methionine *in vivo*. Furthermore, western blotting of these GFP transgenic lines supports the cleavage of GFP from the 5' terminal exon; in line with microscopic analysis suggesting a potential role as a transit signal peptide. Additional investigation in identifying the subcellular localization is currently underway. Future studies will test which of these APT1 isoforms can complement the observed male sterile phenotype, and further identify if these APTs have unique protein interacting partners.

### **2C-5. Redox modulation of starch synthases in wheat and maize endosperm.**

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Redox modulation is a regulatory mechanism used by a large variety of different biochemical pathways. We are currently investigating the potential role that redox modulation has in controlling starch synthesis. Starch is a glucose polymer composed of amylose and amylopectin and is made by the coordinated action of many different enzymes. In this study, the focus was on the soluble starch synthases of which there are four isoforms (I-IV). Evidence suggests that SSI-III function to elongate the growing glucan chain. The function of SSIV is currently unknown but it may be involved in starch granule priming. In this study, the effect of redox modulation on the activity of the starch synthases in wheat and maize was investigated. The results show that chemical reduction causes a large increase in the activity of both the wheat and the maize starch synthases and that the system is reversible. Individual isoforms were investigated to see which isoforms were affected by redox modulation.

## **2C-6. Plants acquired essential functions and diverse mechanisms of metabolic regulation through the evolution of ancient and recent shikimate kinase gene duplicates.**

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Shikimate kinase (SK) catalyzes the phosphorylation of shikimate using ATP towards the biosynthesis of many aromatic compounds required in plants for development, energy processes, and stress responses. Previous studies have suggested that plant SKs act as regulatory control points, facilitating metabolic flux towards specific secondary metabolite pools. Towards understanding the role of plant SKs in metabolic regulation we assess the functional significance of plant SK gene duplicate evolution. The evolution of gene duplicates has been proposed as a central mechanism for the diversification of compounds produced by plant secondary metabolism and the regulation of these metabolic pathways. Using biochemistry, genetics, and bioinformatics approaches we show that SK gene duplicate evolution has yielded novel enzyme functions and mechanisms of metabolic regulation in response to developmental and stress-related requirements. An ancient SK gene duplicate that lost SK activity, shikimate kinase-like 1 (SKL1), is essential for chloroplast biogenesis. Recent plant duplicates retain SK activity and regulate metabolism by diverse gene expression patterns. Biochemical and systems-level knowledge of function among these SK homologs will provide important information regarding plant development and physiology and may yield important insights for the design of enzyme function, antibiotics and herbicides, and metabolic engineering.

## **2C-7. Taxonomic distribution of alternative oxidase in non-angiosperm plants.**

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Alternative oxidase (AOX) is a terminal ubiquinol oxidase that introduces a branch point in the respiratory electron transport chain. The AOX pathway can pass electrons directly to oxygen, bypassing the cytochrome pathway and resulting in less ATP production, with the excess free energy dissipated as heat. AOX is often described as being ubiquitous in the plant kingdom, yet most research has focused only on angiosperms. Our goal was to assess the taxonomic distribution of AOX in non-angiosperm plants. Our preliminary bioinformatics survey indicates that AOX is present in a moss, liverwort, fern, lycopod, and several species of conifers. We are currently confirming the expression of AOX genes using RT-PCR in these species, as well as members of other non-angiosperm plant lineages (e.g. cycads, ginkos, etc.) for which molecular database information is currently unavailable.

### **2C-8. Regulation and localization of arogenate dehydratases in *Arabidopsis thaliana*.**

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In the *Arabidopsis* genome, six genes have been identified, each encoding an arogenate dehydratase (ADT1 through ADT6). ADTs catalyze the decarboxylation/ dehydration of arogenate in the final step of the phenylalanine biosynthesis pathway. All ADTs share a common structure with an N-terminal transit peptide, a core catalytic domain, and a C-terminal ACT or regulatory domain. This overall structural similarity extends to the amino acid sequence level. To characterize this gene/protein family in *Arabidopsis* we are combining biochemical and molecular approaches to understand how each of these enzymes contributes to the system-wide production of phenylalanine. Performing *in vitro* biochemical analyses together with an *in vivo* heterologous yeast expression system allows the differentiation of similar but not identical enzymatic properties. Gene-specific expression studies have defined the tissue-specific expression patterns for each ADT. Furthermore, we have evidence for chloroplast-specific targeting of the *Arabidopsis* ADTs in transient tobacco transformants. Taken together, this provides the first comprehensive analysis of an ADT protein family in plants.



### **P1. NAC transcription factors play a number of roles during age-related resistance in *Arabidopsis thaliana*.**

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As *Arabidopsis* matures it becomes more resistant to virulent *Pseudomonas syringae* pv. *tomato* (*Pst*). This is known as age-related resistance (ARR). ARR requires intercellular accumulation of salicylic acid (SA), and it is associated with flowering. Microarray technology identified genes that were differentially expressed during ARR. ARR assays on T-DNA insertion mutants of some up-regulated genes, including *NO APICAL MERISTEM CUP-SHAPED COTYLEDONS* (*NAC*) transcription factors, demonstrated that they were deficient in ARR. RT-PCR analysis revealed that *NAC1* was expressed earlier during ARR in mature plants compared to basal resistance in young plants, and *NAC2* was expressed at a lower level during ARR compared to basal resistance. Gene expression and *nac1nac2* double mutant analyses demonstrated that the *NAC* genes function in the same ARR pathway. Weekly ARR assays and flowering data revealed that the ARR deficiency exhibited by *nac1* plants was associated with a one week delay in flowering. After *nac1* plants flowered they displayed enhanced resistance to *Pst*. Therefore, *nac1* plants seem to exhibit a one week delay in the onset of an enhanced ARR. *NAC1* may be a positive regulator of the onset of ARR and flowering, but it is a negative regulator of ARR.

### **P2. Characterization of *Arabidopsis* HD2 histone deacetylases in plant development.**

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Histone deacetylases (*HDACs*) are an essential class of enzyme that are required by eukaryotic organisms for the coordinated expression of genes. They therefore play key roles in cell differentiation and organ development through global gene regulation. These enzymes likely function to down-regulate target genes by increasing the strength of DNA/histone interaction by removing the signal to recruit chromatin-remodeling complexes or by lessening steric hindrance. The *HD2* sub-family of *HDACs* class of enzymes are unique to the plant kingdom. This class of enzymes contains four members known as *HD2A*, *HD2B*, *HD2C*, and *HD2D*. Recent studies on *HD2* class resulted in the finding of its regulation by glucose and abscisic acid (ABA) hormone. Here, our research has focused on identifying the connection between *HD2* sub family, glucose and ABA hormone. Our hypothesis is that *HD2* are induced through glucose signaling which then results in the chromatin remodeling of ABA genes and a negative feed back on *HD2* expression. Thus, the implication of the *HD2* sub family in the glucose signalling pathway and ABA biosynthesis was investigated. Our results seem to suggest that *HD2* induction by glucose is not through the usual glucose signaling pathway that involves hexokinase.

**P3. Mechanical properties of soybean protein films as affected by protein compositions.**

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Soybean proteins are appropriate materials for the production of films for industrial purposes. The current work tested the hypothesis that differences in seed storage protein composition among soybean genotypes will significantly affect the functional properties of films produced from them. Soy protein isolates from sixteen soybean genotypes showed significant differences in protein band number and band intensity after analysis by Sodium Dodecyl Sulfate Polyacrylimide Gel Electrophoresis. Films were produced from protein isolates from six genotypes (Harovinton, OAC Huron, OAC Champion, OAC Bayfield, OAC Aytton and OAC Brussels) that showed a variety of compositional differences. They were tested at the 7<sup>th</sup>, 15<sup>th</sup> and 30<sup>th</sup> day after casting and significant differences in tensile strength, elongation %, elastic modulus and water vapor permeability were found among films produced from different protein isolates. Tensile strength (MPa) was significantly and positively correlated with the intensity of 11.6 and 10.4 kDa molecular weights proteins bands present in the extract. Water vapor permeability (gm<sup>-2</sup>24h<sup>-1</sup>) of the films was significantly but negatively correlated with 100.6, 88.0, 49.4, 41.8, 32.3 and 17.6 kDa molecular weight protein bands. The results support the hypothesis that protein composition differences affect the mechanical properties of the films produced from them.

**P4. Mechanisms of aluminum resistance in *Eriophorum vaginatum*.**

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*Eriophorum vaginatum*, a perennial cotton-grass sedge, is colonizing wetlands in Sudbury, ON, where decades of intensive mining resulted in metal-polluted ecosystems. Inhibition of root elongation, a standard assay for Al toxicity, revealed that *E. vaginatum* is more resistant than other species as root growth was inhibited at 1000  $\mu$ M Al, which is ten-fold higher than average inhibition at 100  $\mu$ M for tolerant species. In this study, the mechanisms of resistance to Al in *E. vaginatum* were investigated using the Lumogallion probe and confocal microscopy. In the leaves, Al was found in the vascular bundles and the bundle sheath extensions, and accumulated in the cell walls of sub-stomatal mesophyll. This pattern of Al localization indicates that Al is being transported apoplastically via the transpiration stream and deposited at the sites of water evaporation. Al was also found sequestered in the waxy cuticle, forming ligands with the negatively charged sites of hydrophobic lipids. The presence of Al in the cell walls and leaf cuticles indicates that compartmentalization of Al outside of the metabolically active protoplast might be a part of the mechanism of *E. vaginatum*'s resistance to pollutants.

**P5. Production of hormones by axenic cultures of *Bradyrhizobium japonicum*, *Rhizobium leguminosorum* biovar *viceae* and *Rhizobium lupini*.**

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Legume – rhizobia symbiosis are well known because of their nitrogen fixing abilities that increase plant productivity. However, other lesser-known direct or indirect means of plant growth-promoting rhizobacteria (PGPR) may be in action. Although functional processes remain elusive, especially in field trials – one of the main mechanism through which growth promotion may occur by PGPR is via the production hormones by rhizobacteria. Recent reports have indicated that cultures of *Bradyrhizobium japonicum* can biosynthesize a wide range of hormones including indole acetic acid (IAA), cytokinins (CKs), ethylene, gibberellic acid and abscisic acid (ABA). As well, PGPR appear to vary in the production of these compounds by species and even strain. In this study the production of 18 different CKs as well as IAA and ABA from axenic cultures of *B. japonicum*, *Rhizobium leguminosorum* biovar *viceae* and *Rhizobium lupini* sampled during the exponential growth phase were analyzed by mass spectrometry (LC – MS/MS). A comparison will be made between hormone profiles of these bacteria, each specific to different plant hosts; soybean, pea-lentil-vetch and lupine, respectively. The possible involvement of the production of these hormones in plant growth promotion will also be discussed.

**P6. Stability of IL-10 transcripts affects IL-10 protein accumulation in *Arabidopsis thaliana*.**

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Transgenic plants are one of the most economical systems for large scale production of recombinant proteins for biopharmaceutical and industrial uses. A large number of human recombinant proteins of therapeutic value such as antibodies, growth factors, cytokines and enzymes have been successfully produced in plant systems. The main technical challenge in the field of recombinant protein production is to produce sufficient level of proteins in plants so that the production system is economically viable. Our research aims to identify the factors that control synthesis and accumulation of recombinant protein in plant. A stepwise dissection of control of human immune-regulatory protein IL-10 was carried out using *Arabidopsis thaliana* as a model system. Experiments were conducted to look at the mechanism of recombinant protein production at transcriptional and post transcriptional level. A mutagenised population of transgenic *Arabidopsis* overexpressing IL-10 gene was developed and accumulation of IL-10 was measured. Transgenic plants in the population displayed varying levels of IL-10 accumulation. The transgenic lines that differed significantly at the level of IL-10 protein production were chosen to obtain homozygous lines. The fates of trans-gene in these sets of plants were compared in detail by measuring synthesis and accumulation of IL-10 transcript, transcript stability and IL-10 protein accumulation. The results will be discussed.

**P7. Linking phenotype to genotype in *rosewood*, an activation-tagged poplar mutant.**

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Activation tagging is an insertional mutagenesis technique which results in upregulated transcription levels of an endogenous host gene. To further our understanding of tree genomics we have recently created a *Populus* activation tagged population, from which we have identified a mutant which has rose-coloured xylem named *rosewood*. To date, the location of the T-DNA has been determined using a PCR-based technique, and a candidate gene has been identified using quantitative PCR on the genes neighbouring the insert. To confirm the role of the candidate gene in *rosewood*'s phenotype, we will be transforming wild-type poplar with the gene under the control of a constitutive promoter. We will be also making use of a new technique which takes advantage of the cambial meristem to produce transgenic tissue sectors relatively quickly. To explore *rosewood*'s phenotype at the proteomic level, we will be using two-dimensional gels to detect proteins that are up or downregulated compared to wildtype. Metabolic analysis of the wood revealed the red colour is an anthocyanin, and we hope the proteomic studies will bring to light a link between the candidate gene and the anthocyanin pathways.

**P8. Physicochemical properties of edible films from dry bean genotypes protein.**

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This study was conducted to determine the suitability of dry bean protein as a raw material for the production of biodegradable films. Protein extracts from seeds of 10 different varieties of dry bean were screened by sodium dodecyl sulfate-poly-acrylamide gel electrophoresis (SDS-PAGE) to characterize the types and amounts of proteins they contain. The SDS-PAGE patterns were divided into two regions: Region I, ranging from 58KDa to 201KDa, consisting of 6 monomorphic bands and Region II consisting of 19 bands ranging in size from 11KDa to 56KDa; 9 of which were polymorphic. Based on the varieties of proteins they contain three varieties (including: Hooter, Rex and Elk) were studied to determine how the protein composition affects the functionality of biodegradable films produced from them. Protein films made from Hooter and Rex protein had greater tensile strength and % elongation compared to those produced from Elk. All films possessed similar oxygen barrier properties and a good UV light barrier properties compared to commercial plastic films so they may have applications in packaging applications which are UV sensitive.

**P9. Transcriptome profiling suggests a link between carbon assimilation, photosynthesis and floral induction in maize.**

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We have profiled the molecular differences between normal flowering maize and the severely delayed flowering mutant *indeterminate1*. The INDETERMINATE1 (ID1) transcription factor is a key regulator of the floral transition in maize. *ID1* is expressed exclusively in immature leaves where it controls the production or transmission of leaf-derived florigenic signals. Loss-of-function *id1* mutants have an extended vegetative state; however they exhibit no obvious developmental defects in early growth stages. Oligonucleotide microarray analysis of immature leaves of normal flowering and *id1* mutant plants prior to the floral transition revealed 55 genes with a significant 2-fold difference in expression. Most prominent is a novel family of three maize  $\beta$ -glucosidase genes (*Zmdhr*). These genes are undetectable in *id1* mutants and are expressed in normal immature leaves in a pattern identical to the *ID1* gene. Furthermore, a significant proportion of genes up-regulated in *id1* mutant immature leaves have potential roles in photosynthesis and carbon fixation substantiating a possible connection between floral induction and assimilate partitioning. Finally, the expression analysis of these genes in florally induced vs. uninduced teosinte, a photoperiod sensitive progenitor of day neutral maize, showed no expression differences, suggesting that ID1 acts in an autonomous floral induction pathway that involves novel components.

**P10. Cold acclimated winter cereals exhibit an enhanced potential for CO<sub>2</sub> assimilation under elevated CO<sub>2</sub> conditions.**

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Since cold acclimation of winter rye, wheat and *Arabidopsis thaliana* is associated with enhanced rates of light-saturated CO<sub>2</sub> assimilation with minimal changes in photosynthetic efficiency, we hypothesized that exposure of cold acclimated (5°C) winter cereals (cv Norstar; cv Musketeer) to elevated CO<sub>2</sub> (700 ppm) should stimulate photosynthetic capacity relative to non-acclimated controls (20°C). Furthermore, cold acclimated winter and spring cereals (cv Katepwa; cv SR4A) should exhibit a differential stimulation of photosynthetic capacity upon exposure to elevated CO<sub>2</sub>. Short-term exposures to elevated CO<sub>2</sub> stimulated photosynthetic capacity to a greater extent in cold acclimated winter rye and wheat (80-90%) than in non-acclimated controls (60-70%). However, exposure to elevated CO<sub>2</sub> only compensated for the cold acclimation-induced inhibition of CO<sub>2</sub> assimilation in spring cereals. Although this CO<sub>2</sub>-dependent stimulation of photosynthetic capacity was stable for 6 hours at 40 °C, photosynthetic capacity was inhibited by 80-95% after 80h at 40 °C in all cultivars. We conclude that cold acclimation of winter cereals induces a differential enhancement of CO<sub>2</sub> assimilation compared to spring cereals. Although the CO<sub>2</sub>-stimulated rates of photosynthesis are inhibited by high temperature in a time-dependent manner, elevated CO<sub>2</sub> appears to alleviate initial inhibition of CO<sub>2</sub> assimilation during the first 6h of exposure to high temperature.

**P11. Identification and characterization of a novel calmodulin-binding kinase from soybean root nodules.**

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Ca<sup>2+</sup> ions act as key secondary messengers in most eukaryotic cell signaling pathways, often through the action of the ubiquitous Ca<sup>2+</sup> sensor protein, calmodulin (CaM). CaM is an evolutionarily conserved eukaryotic protein that regulates the activities of many cellular proteins and has recently been shown to be critical for proper initiation and formation of symbiotic nodules in soybean (*Glycine max*). To further our understanding of Ca<sup>2+</sup> signaling in nodule function, we constructed a soybean nodule cDNA expression library and screened it with <sup>35</sup>S- radiolabelled CaM to identify novel CaM targets. In addition to many known CaM targets, we isolated a previously uncharacterized CaM binding kinase (GmCaMK). CaMKs are well studied in animal systems but much less so in plants. Database analyses suggest that orthologs of GmCaMK are present across a range of taxa, including *Arabidopsis*, and widely expressed among tissue types. Initial biochemical characterization has shown that this GmCaMK possess a CaM binding domain at the C-terminus, which binds CaM with high affinity in a Ca<sup>2+</sup>-dependent manner. Current work is focusing on the effect of Ca<sup>2+</sup>/CaM on in vitro kinase activity (K<sub>m</sub>). Biochemical and genetic approaches are underway to identify GmCaMK targets and elucidate the role of this novel kinase.

**P12. Differential expression of *CHS7* and *CHS8* genes in soybean.**

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Chalcone synthase (*CHS*) catalyzes the first reaction specific for flavonoid and isoflavonoid biosynthesis. The reaction involves stepwise condensation of three acetyl units from malonyl-CoA with a 4-coumaroyl CoA to give chalcone. *CHS* gene is present in all plant species. Some plants such as *Arabidopsis* and parsley have a single *CHS* gene in their genome while the soybean genome contains eight copies of *CHS* genes (*CHS1-CHS8*) and a *dCHS1* (duplicate copy of *CHS1*). All members of the *CHS* multi-gene family share a high degree of sequence similarity at the amino acid level within a plant as well as among other plants. However, they play different roles during plant development or in response to environmental stimuli. Comparison of global gene expression in two soybean cultivars that differ in the level of total isoflavonoid accumulation has pointed out the involvement of *CHS7* and *CHS8* genes in isoflavonoid synthesis. In this study, we have extended our effort to understand expression patterns of these two genes in soybean and transformed *Arabidopsis*. We fused a 1.7 kb fragment of the *CHS7* gene promoter and 1.6 kb fragment of the *CHS8* gene promoter with a  $\beta$ -glucuronidase (*GUS*) reporter gene and monitored the *GUS* expression in different tissues of transformed *Arabidopsis*. The results will be discussed.

**P13. Developmental anatomy of *Arabidopsis thaliana*: creating a model for growth stages analysis through a new histochemical technique.**

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The principle growth stages based on phenotypic characteristics on *Arabidopsis* have become a framework for reporting data from various laboratories on a uniform plant material. Thirty growth stages were identified in the development of *Arabidopsis* (Plant Cell, 2001, 13:1499). Our research is complementing this reported growth stage model by defining the internal anatomy. Specifically, we have defined the development of tissue layers of the root, hypocotyl and inflorescence of *Arabidopsis* and correlated the internal anatomy to the external morphology at each growth stage. We developed a new contrasting histochemical technique applicable to free-hand sections. We used TBO in 20% calcium chloride to stain lignified cell walls blue and counterstained primary cell walls with ruthenium red. When mounted in 100% glycerol, hand sections are preserved semi-permanently without losing the intensity and contrast of staining for several months. Such sections could be re-examined if needed for further analysis. Using this technique, we were able to visualize the differentiation of tissues and follow secondary growth in *Arabidopsis*. We created a model of internal *Arabidopsis* development in which internal structure could be predicted from external morphology. Currently, our research is focusing on evaluation of our model using *irx-1*, *irx-3*, *scr-1*, and *iff-1* mutants.

**P14. The tomato bushy stunt virus replication proteins, p33 and p92, in concert with the host-cell ESCRT machinery, are involved in the biogenesis of peroxisome multivesicular bodies in *Saccharomyces cerevisiae*.**

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Peroxisomes are remarkably dynamic organelles that function in a variety of cellular events. They also serve as platforms for the replication of certain viral pathogens. For instance, the tomato bushy stunt virus (TBSV) recruits peroxisomes for its viral RNA replication; a process in which the peroxisome is transmogrified into a peroxisomal multivesicular body (pMVB). While previous results indicate that both components of the TBSV replication complex, namely the 33-kDa RNA-binding protein (p33) and 92-kDa RNA polymerase (p92), are targeted specifically to peroxisome membranes, it is not known whether p33 and p92, either alone, or together, are the minimal viral components required for pMVB biogenesis. It is also unclear what host-cell factors function with p33 and/or p92 in pMVB biogenesis. Using yeast as a model system for TBSV replication, we show that p33 expressed with modified (defective interfering) viral RNA transcripts or p92 expressed alone are minimally necessary for pMVB formation. We show also that the soluble C-terminal portions of p33 and p92 interact with at least two components of the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, Vps23 and Vps28. These and other data support the premise that p33 and p92 appropriate ESCRT to facilitate the formation of pMVBs in infected plant cells.

**P15. The type 8 and 9 glycerol 3-phosphate acyltransferase (GPAT) enzymes are localized to the same ER subdomain, but possess distinct ER retrieval motifs: functional divergence of the dilysine ER retrieval motif in plant cells.**

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Glycerolipids play an essential role in plant biology by serving as major components of cellular membranes, seed storage oils, and the cuticular surface of plant organs. Here, we describe the cellular properties of the enzyme that mediates the initial step in the production of glycerolipids, glycerol-3-phosphate acyltransferase (GPAT). Using the *Arabidopsis* and tung (*Vernicia fordii*) GPAT enzyme families as a model system, we describe the subcellular localization and topological orientation of GPAT8. We also describe the identification of a new putative GPAT, referred to as GPAT9, which we identified through homology-based searches using mammalian GPAT3 and which is a candidate for storage oil formation in developing seeds. Overall, we show that GPAT8 and GPAT9 are both localized to the same ER subdomain and exhibit a similar topological orientation in ER membranes, but that they contain distinct types of ER retrieval sequences. Further characterization of the GPAT8 ER retrieval signal in other members of the GPAT8 family allowed us to expand the functional definition of this signal in plants. Discussed are the implications of these results for the understanding of ER subdomain biogenesis and ER protein trafficking and the regulation of glycerolipid biosynthesis by GPATs located in different subcellular organelles in plant cells.

**P16. An investigation of the exocyst complex, and its role in compatible pollen-pistil interactions in *Arabidopsis*.**

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Vesicle trafficking in plants is essential in numerous processes such as cell expansion, pollen tube elongation and immune response to pathogens. Exocytosis is a highly ordered vesicle trafficking process where vesicles are delivered to the plasma membrane and secreted to the cells exterior. The exocyst is a large eight subunit multimeric complex that is primarily involved in polarized or regulated exocytosis in eukaryotic cells where it functions to tether exocytic vesicles to the plasma membrane. Originally identified in *Saccharomyces cerevisiae*, the exocyst is composed of eight subunits; Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p, and putative homologs have been identified in both animal and plant genomes. In *Arabidopsis*, the majority of the exocyst subunit genes exist as single copies or duplicates, but interestingly, there has been an expansion of the *AtExo70* gene to produce a 23-member gene family. One of the Exo70 members has been recently implicated in pollen-pistil interactions in *Brassica* and *Arabidopsis*. My current research goals are to study the role and localization of the exocyst during pollen-pistil interactions in *Arabidopsis*.



**P17. Validation of *de novo* bioinformatic predictions of *Arabidopsis thaliana* cis-regulatory motifs using *in planta* GFP/GUS expression assays.**

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Cis-regulatory motifs (CRMs) are transcription factor (TF) binding sites that allow for gene activation, enhancement, suppression, or silencing. Currently, CRMs are poorly characterized with < 2% known CRMs for the ~1500 *Arabidopsis thaliana* TFs. Our research aims to characterize *de novo* predictions of CRMs involved in abiotic stress responses and tissue specific expression. The *de novo* predictions were generated using *AtGenExpress* expression compendia and custom designed expression profiles. Due to individual algorithm weakness, a computational pipeline of probabilistic and enumerative methods were used for CRM prediction resulting in putative CRMs for 38 conditions; 20 of which, based on CRM composition and downstream target function, are being further analyzed. The 20 putative CRMs will be Gateway-cloned into eGFP:GUS binary vectors as native, synthetic, or null constructs, and stably transformed into *A. thaliana* Columbia-0. Their activity will then be evaluated under the corresponding condition or tissue type by eGFP/GUS expression levels allowing for verification, validation and characterization of the putative CRMs. Characterization will have significant biological and industrial applications allowing for a better understanding of mechanisms governing genetic regulation; and promoter constructs with spatial and temporal control and predictable expression patterns.

**P18. SLCYSPRO, a Vacuolar Enzyme Potentially Involved in Endosperm Programmed Cell Death is Regulated by Gibberellic Acid and Ethylene.**

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Programmed cell death (PCD) is a fundamental process that is commonly utilized by plants to recover and re-use valuable nutrients from unwanted cells. During and following germination, extensive PCD of the endosperm results in the mobilization and absorption of additional nutrients by the growing embryo. During endosperm PCD specific cysteine proteinases accumulate and become active, thus mobilizing and recycling catabolites. Recently a novel cysteine proteinase, SLCYSPRO, was identified in tomato seeds. To determine if this cysteine proteinase is involved in programmed cell death, tomato seeds were examined for the expression and accumulation of SLCYSPRO. SLCYSPRO protein was shown to accumulate solely in tomato endosperms and its accumulation correlated strongly with increasing levels of PCD. This cysteine proteinase also appeared to be regulated by various plant hormones; in particular we provide evidence that the addition of gibberellic acid and ethylene synergistically promotes both the accumulation of the enzyme and programmed cell death in isolated tomato endosperms. Localization experiments using a 35S::SLCYSPRO-GFP construct indicated that SLCYSPRO is vacuolar. Based on the localization, expression and accumulation patterns of SLCYSPRO it can be suggested that this proteinase plays a role in PCD, however the particulars of that role remain to be determined.

**P19. Quantitative expression analysis for six *Arabidopsis* AROGENATE DEHYDRATASEs in response to heat and cold stress.**

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Six AROGENATE DEHYDRATASE (ADT) genes encode enzymes required for phenylalanine biosynthesis in *Arabidopsis thaliana*. Initial work determined that all six ADTs are constitutively expressed in all *Arabidopsis* tissues, albeit at different levels. As phenylalanine is required for both protein and secondary metabolite synthesis, it has been suggested that the requirement for this amino acid might change in response to environmental conditions. To test this hypothesis, mature *Arabidopsis* plants were subjected to a 24 hour cold (6°C) or heat (38°C) treatment. RNA expression levels were determined at various time points during the treatment and following a 24 hour post-stress recovery using qRT-PCR. In response to cold, expression of all six ADTs increased, either with a single (ADT1, ADT3, and ADT6) or bimodal (ADT2, ADT4, and ADT5) rise in expression. Expression returned to basal levels for all ADTs following a 24 hour post-stress recovery. These expression patterns indicate a role for ADTs as part of the cold response. Conversely, in response to heat stress only ADT2 and ADT4 transcript levels increased, whereas expression of all ADTs increased following a 24 hour post-stress recovery period. These findings are consistent with ADTs not playing a role in the primary heat shock response but being required when the plant is responding and repairing heat damage.

**P20. Comparison of DNA methylation at different times of the day in an early-flowering line of flax (*Linum usitatissimum* L.) and its control.**

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When germinating flax seedlings from pure-breeding genotypes were treated with the DNA demethylating agent 5-azacytidine, many of their first generation progeny had altered phenotypes. Several flowered substantially earlier than normal and subsequent selection produced five pure-breeding, early-flowering lines. The early flowering line RE2 came from the oilseed cultivar Royal (RC). Compared to their control lines all early-flowering lines have a truncated late-vegetative phase, short stature and reduced leaf number. Their total DNA, from various young tissues and early developmental stages, usually has a reduced level of 5-methyl cytosine (5mC) but this induced hypomethylation is often absent in more mature plants or tissues. A recent study of 5mC levels demonstrated that the hypomethylation in RE2 was also absent in total DNA extracted from green tissues of 21-day-old plants that were grown in the dark prior to extraction. Experiments have eliminated the more obvious explanations for this observation. A preliminary experiment has examined the possibility that the presence/absence of the hypomethylation in RE2 is related to the dark period prior to extraction. This experiment, which used total DNA extracted at various times of the day from three tissues of RE2 and RC is described and discussed.

**P21. Regulation of retrograde movement of tRNA from the cytoplasm to the nucleus by nutrient stress may not be universally conserved between yeast, mammals and plants.**

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The intracellular trafficking of tRNA was long thought to be a one-way trip from the site of biogenesis in the nucleus to the translation machinery in the cytoplasm. This view has recently been challenged by the findings that, under conditions of nutrient stress, tRNA can move retrograde from the cytoplasm to the nucleus in the yeast *Saccharomyces cerevisiae* and in rat hepatoma (H4IIE) cells. Contrary to these findings, we have obtained data suggesting the tRNA retrograde process does not occur in response to nutrient stress in several plant and mammalian cell lines, including tobacco and *Arabidopsis* suspension-cultured cells and rat hepatoma (H4IIE) cells. This apparent absence of retrograde tRNA movement from the cytoplasm to the nucleus in plants and mammals may be related to the nuclear localization of the tRNA splicing machinery in these cells, which, unlike *S. cerevisiae*, do not require nuclear export and subsequent re-import of tRNAs during the maturation process. Overall, these findings call into question the universality of retrograde tRNA movement among evolutionarily diverse organisms and suggests instead that this phenomenon may be limited to *S. cerevisiae*.

**P22. Inhibition of *Fusarium graminearum* growth by corn defensin protein expressed in *Escherichia coli* and *Pichia pastoris*.**

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The Plant Defensin gene from corn (*PDC1*) was isolated from genomic DNA with primers designed from a maize EST sequence homologous to a barley defensin. A cDNA sequence corresponding to the defensin exon was synthesized from maize mRNA. The deduced 9 KDa protein, called plant defensin corn 1 (*PDC1*), has the typical features of a plant defensin, including a signal sequence of 35 amino acids, followed by a characteristic defensin domain of 47 amino acids containing 8 cysteines. The defensin protein was expressed from the cDNA clone in *Escherichia coli* and yeast (*Pichia pastoris*) which differ in their ability to catalyze disulphide bond formation that stabilizes the 3-D structure of protein. The nickel column purified *PDC1* preparations showed single peaks of 11 kD and 9 kD for protein with and without His-tag, respectively by MALDI-TOF analysis. *PDC1* protein samples had antifungal activities against *Fusarium graminearum*. Both preparations had antifungal activity but *PDC1* from *P. pastoris* was more effective against *F. graminearum* compared to the protein expressed in *E. coli*. In addition, removal of the His-tag used for purification increased the fungicidal activity of *PDC1*. These data show that the defensin *PDC1* from corn effectively restricts the germination and growth of *F. graminearum*.

**P23. *BLADE-ON-PETIOLE 1* and *2* interact antagonistically with *BREVIPEDICELLUS* and *BELLRINGER* to control *Arabidopsis* inflorescence architecture.**

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*BLADE-ON-PETIOLE1/2* encode two NPR1-like proteins that function redundantly to control the architecture of leaves, flowers, and siliques. Mechanistically, it has been shown that BOP activity controls proximal-distal patterning in leaves in part by excluding the expression of class I homeobox genes in leaf initials. Spatial and temporal regulation of the class I homeobox gene *KNAT1/BREVIPEDICILLUS (BP)* determines multiple aspects of plant architecture, including proximal-distal leaf patterning, leaf serration, pedicel structure, internode elongation, and phyllotaxy. *BPs* expressed in the shoot apical meristem and in inflorescences and functions in a complex with *BELLRINGER (BLR)*, a TALE homeodomain protein. Here, we show that *BOP1/2* function synergistically with *ASYMMETRIC LEAVES1* and *2* to exclude *BP* expression from leaf petioles during vegetative development. We also show that during reproductive development, *BOP1/2* activity opposes *BP/BLR* function to regulate internode elongation, node architecture, and phyllotaxy in inflorescences. Plants overexpressing *BOP1/2* show *bp* and *blr*-like phenotypes whereas inflorescence defects in *bp* and *blr* mutants are rescued in a *bop1 bop2* background. Inflorescence defects in *bp* and *blr* mutants are caused by *KNAT2/6* misexpression. We are therefore testing if mutation of *bop1 bop2* restores normal patterns of *KNAT2/6* expression in *bp* and *blr* inflorescences.

**P24. Phosphatidylglycerol is required for chloroplast biogenesis during cold acclimation of *Arabidopsis thaliana*.**

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A phosphatidylglycerol (PG) deficient mutant (*pgp1*) of *Arabidopsis thaliana* was used to assess the role of PG in the biogenesis and function of the photosynthetic apparatus during cold acclimation. In contrast to wild type (WT), a distinct yellow phenotype was evident during growth of the *pgp1* mutant at 5°C, which was not detected at 20°C. Cold acclimation of the *pgp1* mutant was associated with a significant reduction in the total chlorophyll content and a high Chla/b ratio (5.0 ) compared to either growth of *pgp1* at 20°C (2.6) or the WT ( 2.7). Immunoblot analysis indicated a decreased abundance of Lhcb1 and Lhcb2 polypeptides under these conditions. Electron microscopy revealed a lower number of thylakoids per grana stack in *pgp1* mutant grown at 20°C compared to WT, while the number of stromal thylakoids remained the same. In cold acclimated *pgp1* mutant, the formation of grana stacks was completely inhibited and the number of stromal thylakoids per chloroplast was significantly reduced compared to WT. Concomitantly, 77K chlorophyll fluorescence emission spectra demonstrated significant redistribution of excitation energy in favor of PSI in the *pgp1* mutant regardless of the growth conditions. The role of PG in the structure and function of the photosynthetic apparatus will be discussed.

**P25. Investigation of a putative mobile, long-distance signal that promotes flowering in maize.**

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Higher plants use a multitude of perceptive measures to regulate the floral transition, which involves careful coordination of both environmental and endogenous cues. Recent molecular and genetic analyses in Long-Day (LD) *Arabidopsis* suggest that a long-distance florigenic signal may be the protein encoded by *FLOWERING LOCUS T* (*FT*). *FT* is activated in mature leaves by a photoperiod sensitive protein *CONSTANS* (*CO*) and travels through the phloem to the shoot apex where it interacts with a transcription factor *FLOWERING LOCUS D* (*FD*). Upon interaction with *FD*, a cascade of floral identity genes is activated to promote the transition to flowering. In contrast to *Arabidopsis*, maize is a Day-Neutral (DN) plant; while teosinte, its ancient progenitor, requires obligate Short-Days (SD) to flower. Recent discoveries of *FD* and *FT* homologs (*DLF* and *ZCN*, respectively) in maize support the notion of a universal, long distance florigenic compound that induces floral transition. We are attempting to verify if *FT* and *FD* functional orthologs exist in maize and if the *FT*/*FD* regulatory module functions to promote the transition to flowering. Furthermore, we are comparing the expression of these genes in wildtype vs. *indeterminate1* mutant (severe late flowering) maize in addition to florally induced vs. un-induced teosinte.

**P26. Characterization of suppressor and enhancer mutants of *BREVIPEDICELLUS* in *Arabidopsis thaliana*.**

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The *brevipedicellus* (*bp*) mutant, caused by a loss-of-function mutation in the *KNAT1* homeobox gene, is known to affect stem morphogenesis in *Arabidopsis thaliana*. The effect of the *BP* mutation is more severe in the *erecta* (*er*) background, with reduced internode and pedicel lengths, bends at nodes and downward-oriented pedicels. *BP* and *ER* genes are also required to delineate nodal boundaries and maintain the radial symmetry of stems and pedicels. Our research aims to identify genes acting on the *BP* signaling pathway by utilizing a forward genetics approach. Two mutant screens were performed using *bp/er* and *bp/ER* seeds to allow for the identification of suppressor and enhancer mutants. The *suppressor4* mutant develops moderate length and perpendicularly-oriented pedicels and partially rescues the distal pedicel bulge in flowers, which is absent in the *bp* mutant. The *enhancer3* mutant (*kinky*) was shown to exhibit more severe phenotypes by the appearance of more severe bends at the floral nodes and enhanced achlorophyllous stripes. The results suggest the *SUPPRESSOR4* gene plays a role in inhibiting the development of distal pedicel bulge and influences both pedicel angle and length. The *KINKY* gene, mapped to chromosome 2, might act with *BP* in regulating proper inflorescence development.

**P27. Quantifying the impact of slope aspect position on physiological stress in grasses and shrubs of a northern semiarid grassland during severe drought.**

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Photosynthetic gas-exchange, reflectance, leaf  $\delta^{13}\text{C}$  and stem water D/H were measured in two grasses and two shrubs occurring on both south-facing and north-facing slopes of a temperate grassland. Response to drought varied between functional types and slope aspects. Grasses (*Stipa viridula* and *Agropyron cristatum*) exhibited high net photosynthesis ( $A_{\text{max}}$ ) in May, but shut down in June, except in north-facing *S. viridula*. The photochemical reflectance index (PRI), chlorophyll index (CI) and NDVI also decreased sharply in June, except in north-facing *S. viridula*. In the shrub species (*Artemisia cana* and *Rhus trilobata*),  $A_{\text{max}}$  decreased in close association with vapour pressure deficit and leaf temperature, remaining positive throughout the growth season only on the north-facing slope.  $A_{\text{max}}$  was near zero during drought in south-facing specimens, but increased after September rainfall. D/H ratios were higher in *A. cana* than in *R. trilobata*, which suggests reliance on distinct water source depths. Higher  $\delta^{13}\text{C}$  and photosynthetic water-use efficiency were observed in south-facing shrubs. NDVI and CI varied little with DOY in *A. cana*, due to its reflective leaf hairs, but PRI showed a marked decrease. In *R. trilobata*, PRI and CI exhibited a parabolic pattern, while NDVI peaked in June.

**P28. Potential roles of *Em* (*LEA1*) protein in the increased salt-tolerance in transgenic tobacco plant.**

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*Em* gene was transformed into tobacco plant, PCR and RT-PCR results indicated that the *Em* gene was successfully integrated into the genomic DNA of transgenic tobacco plants and also expressed at RNA level. The transgenic tobacco seedlings showed better growth performance and higher rooting rate than the non-transformants. To find out the potential roles of *Em* gene in the increased salt-tolerance in transgenic tobacco plant, proteomic approaches were used. Soluble proteins of leaves were extracted from three-week-old tobacco seedlings that were treated with 1.5% NaCl. 2-D gel results showed that three protein spots were expressed most differently between the transgenic tobacco and non-transformants, which are phosphoglycerate kinase-like (PGK), ATP synthetase CF1 beta subunit, and an unnamed protein. Phosphoglycerate kinase is involved in glycolysis in cytoplasm, and is also involved in the Calvin cycle during photosynthesis; ATP synthase participates in oxidative phosphorylation and photosynthetic phosphorylation, it is the core of organisms of energy conversion. Under salt stress, the up-regulated expression of phosphoglycerate kinase and ATP synthase could promote photosynthesis. These results provide clues for understanding the mechanism of protective function of *Em* protein under stress conditions.

### **P29. The lace plant: transformation.**

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The lace plant, *Aponogeton madagascariensis* is an aquatic plant which forms perforations in its leaves as a part of its normal developmental growth. This process of perforation formation is extremely regulated and has been shown to be orchestrated by developmental programmed cell death (PCD). To better understand the mechanisms of PCD which lead to these perforations I am developing a reliable protocol for both transient and stable transformation utilizing the cytosolic construct GFP-pEGAD. In an effort to stably transform lace plant tissue I am utilizing this construct in the following two approaches: 1) immersing leaves, corms, root, and flowers in *Agrobacterium tumefaciens* 2) callus induction from lace plant tissue for transformation. Upon the successful integration of the GFP-pEGAD construct I will utilize more specific constructs to observe changes in the cytoskeleton, tonoplast and the plasma membrane of the lace plant throughout PCD. I am also attempting transient transformation through the isolation of lace plant protoplasts and the utilization of a novel PEG-calcium transfection technique. Results from my preliminary transformation experiments have shown some transformed cells. However, further experiments are required in order to perfect protocols and increase transformation efficiency.

### **P30. Molecular and phylogenetic characterization of related N-methyltransferases from *Arabidopsis thaliana*.**

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Choline in plants is found as free choline and as phosphatidylcholine. In plants studied to date, choline synthesis occurs via a phosphobase route involving water-soluble intermediates. In *Arabidopsis*, phosphoethanolamine-N-methyltransferase (PEAMT designated AtNMT1 associated with At3g18000) has been biochemically shown to catalyze three N-methylations of phosphoethanolamine to yield phosphocholine, the precursor to choline. *Arabidopsis* has two other PEAMT-like enzymes: AtNMT2 (At1g48600) which catalyzes two of three N-methylation reactions and AtNMT3 (At1g73600) whose biochemical activity is unknown. All three genes encode products ca 500 amino acids long containing two potential catalytic domains. AtNMT2 and 3 have 86.6 and 85.1% sequence identity to AtNMT1, respectively. The phylogenetic relationship of these gene products to related sequences was compared via maximum parsimony analysis. The tree shows AtNMT3 is more related to other plant PEAMT genes than either AtNMT1 or 2 and AtNMT2 branches away from other plant PEAMTs including AtNMTs1 and 3. Homology modelling was used to construct three-dimensional models of the two catalytic domains of each AtNMT and shows an alternative folding pattern in one of the catalytic domains of AtNMT2. This offers an explanation why AtNMT2 only catalyzes two of the methylations and suggests AtNMT3 catalyzes all three reactions.

**P31. *Eriophorum vaginatum*: senescence patterns within the vegetative and flowering  
corms**

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*Eriophorum vaginatum* is perennial sedge with a growth habit consisting of interwoven leaf bases, adventitious roots, corms with vertical rhizomes, together forming a tussock. Yearly senescence of vegetative tissues, subsequent regrowth and vegetative reproduction (formation of axillary cormlets) or reproductive (formation of the inflorescences), creates an elevated growth form. Dissections of the mature tussocks revealed extensive senescence of the tillers that contained corms. In the present study, cell vitality was determined by taking free-hand sections of the corm just below the apex, at the middle and at the distal regions of vegetative and flowering corms, and assessed using DAPI, Evan's Blue and Fluorescein. In vegetative corms, senescence was initiated at the distal region and progressed upwards to the apex. In the flowering corms, senescence progresses from apical region downwards, with an observed formation of a lignified and suberized cell layer between the dead and living tissue. However, in corms arising from the top of vertical rhizomes, a suberized protective layer was formed at the rhizome/corm interface. Therefore, the elevated habit of the *E. vaginatum* tussock is due to the progressive growth of new corms, which are connected below to the senescencing older corms experiencing little decay in cold anoxic wetlands.

**P32. The utilization of stored and newly-synthesized mRNAs during seed germination.**

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To find out if newly synthesized mRNAs are necessary for seeds to complete germination, we tested the effects of  $\alpha$ -amanitin on this. Four species were used: Arabidopsis mutant (*tl-2*) seeds, tomato seeds, lettuce embryos and soybean axes. Lettuce embryos achieved a similar percentage of germination in 0.5mM  $\alpha$ -amanitin as in water, but germination was slowed down at a concentration of 0.08mM. Germination was completely inhibited by  $\alpha$ -amanitin at different concentrations in the other three species. RT-PCR time course results indicated when  $\alpha$ -amanitin successfully penetrated into the nuclei and started to inhibit mRNA production. Messages present in the dried seeds or axes had a limited stability, in all species, of around 10 hours. If  $\alpha$ -amanitin started to function at a stage when the time needed to complete germination was shorter, seeds could complete germination. Otherwise seeds or axes did not complete this process. Data from protein gels and western blots show that there is constant production of some proteins from stored and newly synthesized mRNAs that may be required during seed germination. The results suggest that neosynthesized mRNA is needed for seeds to complete germination, and that stored messages alone are not sufficient to support this process.



### **P33. Identifying Vitaceae inducer of CBF expression (ICE) genes.**

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The ability to cold acclimate is a quantitative genetic trait resulting from the input of *many genes*. The freezing tolerance pathway involved in cold acclimation is initiated by a cold stimulus resulting in the activation of a protein called inducer of CBF expression (ICE). The ICE protein is then able to bind and activate the promoter region of the C-repeat binding factor (CBF) genes. Eight potential CBF genes have been identified in grape plants (*Vitis* sp.). Preliminary results indicate that at least 4 *Vitis* CBFs are cold inducible *in planta*. Based on sequence homology to *AtICE1*, 4 *Vitis* ICE genes have identified. The stability of the *AtICE1* proteins is thought to be affected by phosphorylation, sumoylation and ubiquitination, and these modification sites have putatively been identified within the *Vitis* ICE proteins. *In silico* ICE protein and CBF promoter sequence analyses have been performed to determine conserved regions that would classify proteins as ICE and to identify potential ICE binding sites. To determine the activity and specificity of the *Vitis* ICE genes a transactivation assay by means of a dual luciferase system will be used, the protocol to be employed will be outlined.

### **P34. Identification of potential post-transcriptional modification events that regulate the grape transcriptome in response to stress.**

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Abiotic stresses cause a change in the transcriptome and, as result, a change in various compounds that aid the plant to tolerate the stress. The change in the transcriptome can be due to a change in the activity and/or localization of proteins that regulate transcription by changes in their phosphorylation, ubiquitination and/or sumoylation state upon exposure to stress. However, it has become more and more clear recently that other regulatory mechanisms such as alternative splicing and RNA processing, as well as silencing, also play a major role in shaping the transcriptome. My lab investigates the molecular basis of freezing tolerance in grapevines. As part of our quest for molecular markers for freezing tolerance we have identified several putatively stress-related genes for each of its 19 chromosomes. We have determined for one of these genes, encoding a dehydrin, that the intron is retained in part of its transcripts if grape plants are exposed to cold. To investigate whether post-transcriptional modifications are also possible for a high percentage of the other stress-related genes, we have started with their *in silico* analysis and found that it is indeed likely that several of their transcripts undergo alternative splicing.

**P35. Identifying *Pseudomonas syringae* Type III effector proteins that modulate auxin signaling in *Arabidopsis thaliana*.**

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Plant hormones act in a complex network where their pathways regulate and interact with each other to control different responses. This cross talk between hormones can be exploited by pathogens to suppress plant defense responses and thereby increase their growth. *Pseudomonas syringae* pathogenicity is reliant on a Type III secretion system (TTSS) that acts as a specialized injection apparatus to deliver virulence proteins, known as type III effectors (TTEs), into the plant cell cytosol. A few well characterized TTEs, such as AvrPto2, HopAM1 and AvrPto, have been shown to modulate *Arabidopsis thaliana* hormone signaling pathways. In this study, we have screened hormone promoter::uidA transgenic *Arabidopsis thaliana* lines against a *P. syringae* TTE library in order to identify TTEs involved in the perturbation of hormone signaling *in planta*. The screening follows a quantitative and a qualitative approach using different transgenic *Arabidopsis* GUS lines. We have identified three *P. syringae* TTEs, capable of inducing auxin signaling using transgenic IAA1::uidA or DR5::uidA seedlings exposed to these TTEs. Whether these effectors are directly or indirectly manipulating the auxin signaling pathway remains to be elucidated.

**P36. Alleviation of low temperature sweetening in potato by overexpressing *Arabidopsis thaliana* Pyruvate decarboxylase 1.**

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Potato tubers stored at temperatures below 9-10°C result in high concentrations of reducing sugars such as glucose and fructose known as low temperature sweetening (LTS). These reducing sugars participate in the Maillard browning reaction with free amino acids during frying resulting in dark-brown coloured fries and chips, which are unacceptable to consumers. Our earlier research with a LTS-tolerant and LTS-susceptible variety has shown that, ethanol and lactate levels are higher in LTS-tolerant variety and were strongly correlated to improved chip color. As well, a positive correlation was observed between reducing sugar concentration and the  $K_m$  of pyruvate decarboxylase (PDC), with the LTS-tolerant ND 860-2 possessing a lower  $K_m$  and reducing sugar content than the LTS-susceptible Monona. These results suggest a role for PDC in LTS-tolerance. To test the role of PDC, a popular potato variety, Snowden, used for the production of potato chips, was transformed with *Arabidopsis* cold-inducible pyruvate decarboxylase gene 1 (APDC1) under the control of cold-inducible promoter rd29A. The insertion of APDC1 gene and its expression in the two transgenic potato plants selected were confirmed by Southern and Northern analyses respectively. Experiments are underway to characterize the LTS-tolerance of the transgenic tubers.

## **P37. Transcriptional regulation and downstream targets of AtMYB61.**

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AtMYB61, a member of the R2R3-MYB family of transcription factors in *Arabidopsis thaliana*, modulates gene expression in response to diurnal cues to control the major facets of the plant transpiration stream, namely, diurnal changes in stomata aperture, xylem formation and root system architecture. AtMYB61 also alters gene expression in response to sugars, resulting in modification of plant architecture and cell wall structure. AtMYB61 transcript abundance increases to the greatest extent in response to the major product of photosynthesis, sucrose, and is repressed in response to two major products of photorespiration, glutamate and glycine. AtMYB61 expression is de-repressed by soluble sugars in a mechanism that involves intragenic sequences. Phylogenetic footprinting, bioinformatic, and biochemical analyses suggest a role for intron sequences in the regulation of AtMYB61 expression. Recently, three putative downstream target genes of AtMYB61 were identified. The three putative targets of AtMYB61 were predicted on the basis of comparative transcriptome analyses between microarrays that examined gene expression changes that were modulated by difference in AtMYB61 activity and sugar and those that examined the co-expression of AtMYB61 across development and in different organs. Statistically over-represented motifs were identified in the 5' non-coding regions of the three putative target genes, and these correspond to previously characterized AC element motifs that function as R2R3-MYB targets. The consensus motif functions as a *bona fide* target for AtMYB61 binding as determined by an electrophoretic mobility shift assay. Binding between the gene regulatory sequences of the putative target genes, which contain multiples of these motifs, was confirmed via electrophoretic mobility shift assays.

## **P38. Differential gene expression in *Arabidopsis thaliana* in response to infection by *Fusarium graminearum*.**

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The fungal pathogen *Fusarium graminearum* infects a variety of cereals, including wheat, corn, and barley resulting in significant economical losses, both in Canada and abroad. The pathogen accesses nutrients in a variety of ways, including by invasion of vascular tissues. The molecular mechanisms underlying *Fusarium* virulence in cereals is not well understood, largely due to the genetic intractability of these crop plants. The model plant species *Arabidopsis thaliana* is a non-host to this pathogen. However, certain mutants of *Arabidopsis* such as those involved in salicylic acid signalling permit *Fusarium* to germinate and proliferate intracellularly. This opens up the possibility of using *Arabidopsis* as a model organism to understand *Fusarium* pathogenicity. In our effort to understand *Fusarium* ingress, we have identified a mutant of *Arabidopsis* called *cer3* that allows *Fusarium* to proliferate in the vasculature of *Arabidopsis*. *Cer3* encodes a putative enzyme important for the production of waxes associated with the cuticle, which is a protective lipid-based coating on the aerial surfaces of all land plants. DNA microarray studies were performed to identify genes that are differentially regulated in *cer3* plants compared to wild-type in response to infection with *Fusarium*. Genes that are differentially expressed are likely involved in resisting *Fusarium*'s proliferation in the vasculature. These studies in a non-host system will allow insights into the nature of *Fusarium* ingress into the vasculature of host plants.

**P39. Post-translational regulation of ER membrane-bound plant fatty acid desaturase enzymes.**

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ER-localized fatty acid desaturases (FADs) play a central role in plant lipid metabolism by providing polyunsaturated fatty acids for both cellular membranes and storage oils. Here we summarize experiments aimed at elucidating the post-translational regulation of *Arabidopsis* FAD2 and tung (*Vernicia fordii*) and *Brassica* FAD3 expressed in yeast cells. We show that modifications to the N terminus of FAD2 by the addition of various epitope tag sequences substantially increase enzyme activity and steady-state protein abundance in comparison to the untagged, native enzyme. On the other hand, RNA blotting and protein half-life analyses indicated that the amount of tagged FAD2 mRNA and the rate of protein degradation were similar to that of the native protein, suggesting that the N-terminal modifications of FAD2 increased steady-state protein amounts and corresponding enzyme activity by improving translational efficiency. N-terminal epitope-tagged FAD3 enzymes exhibited a similar increase in activity and steady-state protein amount. Furthermore, FAD3 proteins were significantly affected by cultivation temperature, a process that appears to be mediated by a *cis*-acting protein-half-life signal located within their N termini and the ubiquitin-proteasomal degradation pathway. Preliminary experiments aimed at using the photoconvertible Eos fluorescent protein to monitor FAD protein turnover in living plants cells are also presented.

**P40. *Populus* drought transcriptome: Spatial and temporal variation in transcriptome activity.**

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The genus *Populus* provides an excellent opportunity to investigate questions related to the interplay between an individual's environment and its response to external stimuli. *Populus* trees are frequently clonally propagated in multiple locations, each with their own local environment. We are capitalising on this feature of *Populus* trees to test that hypothesis that identical *Populus* genotypes (clones) propagated in different nurseries differ in their drought response at the level of the transcriptome, which in turn influences whole plant phenotype. To test this hypothesis, three commercially favoured hybrid *Populus* clones were each obtained from two different locations and grown under common garden conditions. At the onset of a significant difference in stomatal conductance between well-watered and water-limited plants, leaves were harvested at two time points for transcriptome analysis using whole genome poplar GeneChip microarrays. Bioinformatic analyses revealed dynamic transcriptome remodelling within the same clone based on nursery location and time of day. The results of these experiments provide insights into the interplay between genotype and environment, and hint at the potential role played by epigenetic phenomena in this interaction. Moreover, the results provide mechanistic insights into long-standing questions related to nursery source and future performance of *Populus* clones.

### **P41. Characterization of corn cellulose fiber for manufacturing automotive plastic parts.**

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Corn production in Ontario provides a large source of natural fiber that might be used in automobiles as replacements for glass fibers in plant-based or petroleum-based plastics. However, the use of corn fiber reinforced polymeric composites is limited due to a lack of information about their functional performance properties, especially after freezing and thawing. The objective of the current project is to determine the relationships between the genetic makeup of corn varieties, their fiber compositions and functional properties. Quantitative trait loci (QTL) for cellulose, lignin, and hemicellulose content as well as QTL for corn stalk fiber composition (especially ferulic acid content) will be determined using a recombinant inbred population that is segregating for ferulic acid content in the seed. A protocol for extraction of phenolics from corn stalks and cobs was standardized. Preliminary results show that the parents differ in the free phenolic contents of their stalks and the recombinant inbred population is segregating for free phenolics in their stalks and cobs. The study will provide an understanding of the genetic control of cell wall traits that are important for the use of corn fibres in biocomposite materials. This could lead to the creation of corn varieties in which the cellulose is more easily extractable as pure filaments and therefore more valuable for biocomposite production.

### **P42. Excitation pressure controls the development of variegation in *Arabidopsis thaliana*.**

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Since variegation in the *Arabidopsis thaliana immutans* mutant can be completely suppressed by growth under low light, we hypothesized that excitation pressure (EP) governs the extent of variegation in *immutans*. To test this, we developed an imaging technique to quantify variegation *in vivo*. The plants were grown at either 25°C or 12°C with increasing irradiance (50, 150 and 450  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ). As growth irradiance increased, the extent of variegation increased in *immutans*, as well as in several other variegated mutants (*var1*, *var2*, *atd2*). *immutans* grown at 12°C exhibited greater variegation than *immutans* grown at 25°C at all light intensities. The extent of variegation was positively correlated ( $r^2 = 0.759$ ) with an increase in EP. Structural and functional analyses indicated that thylakoid membrane biogenesis and assembly were inhibited under high EP in *immutans*. WT plants displayed a significantly lower EP compared to *immutans* during early stages of chloroplast development. These results support the thesis that the variegated phenotype is controlled by cellular energy imbalances. We conclude that the lack of IMMUTANS is necessary but not sufficient to account for the extent of variegation in several mutants of *Arabidopsis*. Rather, it is the EP experienced during early chloroplast development that governs the patterns of variegation.

**P43. Phytoremediation of a chemical plume containing 1,4-dioxane.**

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The use of phytovolatilization to remediate environmental contamination is an emerging and green technology in which contaminants are taken up into a plant and are transpired into the atmosphere where they are then degraded. The suspected carcinogen, 1,4-dioxane, is used as a chlorinated solvent stabilizer. It is a heterocyclic organic compound with the molecular formula C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> that is quickly degraded by UV radiation. The concentration of 1,4-dioxane from the transpirant was determined in order to investigate the effectiveness of *Populus balsamifera*, *P. deltoides* x *nigra* (DN34), *P. nigra* x *maximowiczii* (NM1), and *Salix nigra*, in removing the contaminant. The amount of this chemical that is transpired per day by trees of each aforementioned species was calculated and is reported. Comparisons between the tree lines effectiveness in removal of 1,4-dioxane are made. These results will allow for more effective future planning of phytoremediation sites contaminated with 1,4-dioxane or similar contaminants.

**P44. Dissection of the AtMYB61 regulatory circuit by chemical genetics.**

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*Arabidopsis thaliana* MYB61, a member of the R2R3-MYB family of transcription factors, is involved in the regulation of phenotypic plasticity in response to environmental cues, such as light and sucrose availability. AtMYB61 regulates several aspects of plant growth and development, including vascular architecture, secondary root formation, and stomatal aperture. Though AtMYB61 has been shown to play an important role in the regulation of these traits, components of the regulatory circuit that reside upstream of this transcription factor remain to be elucidated. We employed a chemical genetics approach to dissect upstream regulatory elements in the AtMYB61 pathway, involving a screen in search of compounds that differentiated between wild-type seedlings and seedlings containing a *myb61* loss-of-function mutation. Five different chemicals belonging to the sulfonamide family of compounds had a deleterious effect on dark-mediated hypocotyl elongation in wild-type seedlings, while the *myb61* mutants were relatively unaffected. Here we report on the initial chemical screen, and the progress we have made toward using the screen to characterise additional components of the AtMYB61 regulatory pathway. We propose that the sulfonamide insensitivity found in *myb61* seedlings can be used as a starting point to dissect the molecular components involved in the upstream regulation of AtMYB61 activity.

**P45. Within the newly re-classified Plantaginaceae are Iridoids merely secondary metabolites?**

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In the ornamental *Antirrhinum majus* L. and the common weed, *Plantago lanceolata* L. both now re-classified within the Plantaginaceae we have observed that a significant amount of newly fixed carbon is allocated to complex carbohydrates such as monoterpenes (iridoids). In addition to starch and sucrose, *A. majus* and *P. lanceolata* both produce alcohol sugars. While manitol in *A. majus* is not heavily  $^{14}\text{C}$  labeled in comparison to sucrose and starch, the alcohol sugar, sorbitol, in *P. lanceolata* is heavily labeled during  $^{14}\text{CO}_2$  feeding and appears to accumulate in sink tissues. *A. majus* contains two iridoids: antirrhinoside which has been found to be phloem-mobile and accounts for 15 to 24% of total carbohydrates transported depending on the environmental conditions, while the less-mobile antirrhide, which is also heavily labeled accumulates in the laminar tissue. Different environmental conditions such as light and temperature alter  $^{14}\text{C}$ -partitioning and export of these metabolites in *A. majus* cultivars. Interestingly, *P. lanceolata* contains two different iridoids, catalpol and aucubin, which have a similar chemical structure and possibly function analogous to those of antirrhinoside and antirrhide, respectively. In *P. lanceolata* these intermediates are heavily labeled in addition to sucrose and sorbitol. Steady-state  $^{14}\text{CO}_2$  labeling is being used to compare photosynthesis, gas exchange,  $^{14}\text{C}$ -partitioning, and export at varying temperatures, light conditions, and carbon dioxide levels within members of the Plantaginaceae. However, even at this stage in our investigations it seems clear that the early incorporation of newly fixed C within the iridoid pools points to a primary role for these metabolites as photosynthetic products.

**P46. Altered gravitropism, amyloplast sedimentation and circumnutation in *atidd15/sgr5* mutants are associated with reduced starch levels.**

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The *INDETERMINATE DOMAIN (IDD)* genes encode a plant-specific family of putative zinc finger transcription factors. The founding member of the *IDD* family, *INDETERMINATE1 (ID1)* controls flowering time in maize. The *Arabidopsis* genome contains 16 *IDD* genes, which appear to have diverse roles in development. *AtIDD15* is allelic to the *SHOOT GRAVITROPISM 5* locus, loss-of-function of which causes a reduced gravitropic response in *Arabidopsis* inflorescence stems. Gravity-sensing amyloplasts in the shoot endodermis of *atidd15* mutants, sediment more slowly than wild type suggesting a defect in gravity perception. This is correlated with lower amyloplast starch levels, which may account for the reduced sensitivity to gravity in *atidd15* mutants. Further, we find that *sgr5* mutants have a severely attenuated stem circumnutation movement. *adg1-1* and *sex1-1* mutants, which contain no starch or increased starch, respectively, also show alterations in the amplitude and period of circumnutation. Together these results suggest that plant growth movement may depend on starch levels and/or gravity sensing. Overall, we propose that loss of *SGR5* regulatory activity affects starch accumulation in *Arabidopsis* shoot tissues which causes decreased sensitivity to gravity and diminished circumnutation movements

**P47. A KDEL-tailed cysteine proteinase associated with programmed cell death in post-germinative tomato endosperm.**

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Programmed cell death (PCD) is an active process that removes cells, tissues, or organs that are not required for subsequent development. Tomato seeds contain a nutritive endosperm that undergoes PCD following germination and reserve-mobilization to provide additional nutrients for seedling establishment. Prior to cell death PCD-specific organelles, ricinosomes, accumulate in endosperm cells. Upon vacuolar collapse, the point of cell death, these organelles degenerate releasing their contents into the cytosol. We have characterized the expression and accumulation of a KDEL-tailed cysteine proteinase, SICysEP, in germinating and post-germinative tomato seeds. Using this enzyme as a molecular marker, viability assays, and ultrastructural observations we demonstrate that the immature form of the enzyme accumulates in ricinosomes inside endosperm cells prior to, and during reserve mobilization while the endosperm cells are still viable. The mature form of the enzyme is detected as some cells display ultrastructural changes associated with PCD, while other cells are already dead and crushed, and as the viability of the endosperm is decreasing. In this system the signal(s) for producing ricinosomes and marking cells for death remains unclear; however, we demonstrate that exogenous ethylene can signal for the rapid processing of SICysEP, and for the execution of PCD.

**P48. Ectopic expression of an F-Box protein alters inflorescence architecture**

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In *Arabidopsis*, the F-box gene family encodes a large number of proteins, most of which have not been characterized, but are postulated to act as substrate selectors for proteasome-mediated protein degradation. Many recent reports document the importance of various F-box proteins in developmental and metabolic signaling. Our interest in these proteins stems from microarray analyses of inflorescences of wildtype and *brevipedicellus* (*bp*) mutants. In *bp*, several F-box proteins are upregulated, suggesting that BP represses these genes in wildtype plants to condition normal inflorescence development. We therefore undertook analyses to examine the function of these proteins and of how they might contribute to the pleiotropic phenotypes of the *bp* mutant. Yeast-2-hybrid screens revealed that one of the F-box proteins binds to phenylalanine ammonia lyase 1 (PAL1), the gateway enzyme of the phenylpropanoid pathway. These studies are currently being complemented by PAL enzyme assays on F-box mutants and overexpression lines in addition to wildtype and *pal1* mutants. Transgenic lines in which the BP promoter drives F-box expression exhibit defects in phyllotaxy, which is manifest as alterations in axillary branch angles and as the emergence of inflorescence meristems in addition to floral meristems in the axils of some cauline leaves.



**P49. FAR4 and FAR5: Fatty acyl-CoA reductases from *Arabidopsis thaliana* that generate fatty alcohols associated with suberin deposition.**

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Fatty Acyl-CoA reductases (FARs) catalyze the formation of primary fatty alcohols from fatty acyl-CoAs. An eight-member family of alcohol-forming FARs has been identified in *Arabidopsis thaliana*. One of these genes, *CER4* (*At4g33790*), is responsible for the production of very-long-chain fatty alcohols present in the waxy cuticle of the aerial plant surface. Two other genes of the Arabidopsis FAR family, *FAR4* (*At3g44540*) and *FAR5* (*At3g44550*), were found to be highly expressed in root tissues by inspection of DNA microarray data. Using promoter-reporter gene fusions, we found that these two genes are specifically expressed in the cell layer surrounding the central vasculature of the root. These genes were also found to be expressed in response to mechanical wounding. Suberin and associated waxes (e.g. fatty alcohols) are deposited in the wound periderm, casparian strips and the endodermal cell walls of the primary root, which forms a protective hydrophobic barrier that limits water and solute loss from the central stele of roots. Thus, these enzymes are likely responsible for the production of fatty alcohols associated with suberin in roots. The substrate specificities of the FAR enzymes were investigated by measuring the accumulation of fatty alcohols in yeast heterologously expressing *Arabidopsis* FAR enzymes, wherein, FAR4 resulted in accumulation of C20 fatty alcohols and FAR5 resulted in accumulation of C16, C18 and C20 fatty alcohols with preference to C18 alcohols. We are studying the chemical phenotypes in the roots and seed coat of T-DNA knockout mutant lines of each of these genes, which may reveal important roles of fatty alcohols in these specific cell types.

**P50. *Arabidopsis thaliana* (L.) Heynh. having altered expression of mitochondrial pyruvate dehydrogenase kinase show enhanced oil biosynthesis under elevated CO<sub>2</sub>.**

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Our study is based on the hypothesis that *Arabidopsis* transgenic lines with increased dark respiratory rates due to antisense repression of mitochondrial pyruvate dehydrogenase kinase (mtPDHK) are able to show enhanced growth rates and productivity under elevated CO<sub>2</sub> levels compared to control lines due to greater sink capacity. Wild-type and transgenic lines having antisense mtPDHK expression were grown under ambient (380ppm) or high (800ppm) CO<sub>2</sub>. Growth analyses revealed increased height and total length span of inflorescences, increased numbers of siliques and seeds and increased seed weight in transgenics under high CO<sub>2</sub> compared to controls. Analyses of fatty acid profile and oil content of seeds showed enhanced fatty acid and oil content in transgenic lines under high CO<sub>2</sub>. Significant increases in harvest indices seen in transgenic lines grown under elevated CO<sub>2</sub> levels indicated an increased capacity to utilize photosynthates more efficiently at high CO<sub>2</sub> conditions. Interestingly, the best overall improvement in productivity under elevated CO<sub>2</sub> was shown by constitutive lines YA5-10<sup>4</sup> and YA5-3<sup>1</sup> with relatively moderate decreases in mtPDHK expression levels and moderate increases in dark respiratory rates. The importance of dark respiration in maintaining source-sink balance in plants grown under elevated CO<sub>2</sub> will be discussed.

**P51. Activation tagging to identify new genes responsible for trichome development in *Arabidopsis thaliana*.**

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Trichomes, commonly known as plant hairs, are modified epidermal cells which protrude from the leaf surface. Trichomes may play a role in protecting plants from pest invasion, from UV light damage, and increase cold tolerance by maintaining a boundary layer of air around the leaf surface. Cell morphogenesis is regulated by a complex network of gene regulation. Our research aims to characterize genes responsible for trichome development using an activation-tagged *Arabidopsis thaliana* population. We have screened about 20,000 lines and have identified 13 distinct trichome mutants that include alterations in trichome branch number, cell shape, cell initiation and cell surface texture. Sequencing of the genomic DNA surrounding the activation tagging vector was performed on 4 of these mutants, and two mutants appeared to be novel. Further sequencing is underway for the remaining mutants. Based on the diversity of phenotypes found, these results reveal that activation tagging is a valuable method to create new mutants for understanding plant development.

**P52. Molecular characterization of key genes for folic acid synthesis in common bean.**

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Common beans (*Phaseolus vulgaris*) are an excellent source of dietary folic acid, which plays an important role in preventing neural tube disorders in newborns and helps to prevent heart disease and cancer. However, levels of this compound can vary more than three fold among bean varieties. Previous results showed that high levels of folic acid content in bean varieties are correlated with high levels of expression of aminodeoxychorismate synthase (ADCS) and dihydroneopterin aldolase (DHNA) in the folate synthesis pathway. A fragment of the ADCS gene was used as a probe to screen BAC libraries of common bean cultivar OAC Rex and cultivar G19833. Positive clones were identified from OAC Rex library and will be used to transform common bean and *Arabidopsis* to confirm the biological function of the gene. Five positive clones were identified from G19833 library. Based on the physical map available in the Legume Information System (LIS) database, these clones belong to a contig which was mapped on chromosome D (2). The positive clones will be sequenced to characterize the full length ADCS gene in *Phaseolus*. This information will be useful for developing new tools to screen for bean varieties with enhanced levels of folic acid.

**P53. Molecular mapping of genes involved in the phenylpropanoid pathway in common bean (*Phaseolus vulgaris* L.)**

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Previous genetic analyses identified 15 genes that control seed coat pattern and color in common bean (*Phaseolus vulgaris* L.) and some of them have been positioned on the common bean linkage map. It has been hypothesized that genes involved in the phenylpropanoid pathway correspond to some of the classical seed coat color genes in bean. In a previous study we cloned and sequenced fragments of thirty-five phenylpropanoid pathway genes from common bean. The purpose of the current work is to map the positions of these genes on the common bean linkage map and determine whether their position correspond to any of the loci for classical seed coat color genes. The mapping population that was used consisted of recombinant inbred (RI) lines derived from a cross between 'BAT 93' and 'Jalo EEP558'. Polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) were identified for the phenylpropanoid gene sequences between parental lines. The segregation patterns of 20 phenylpropanoid pathway genes have been analysed in the RI population and their locations in the bean linkage map were determined by a JoinMap analysis. The additional genes in this pathway will be mapped in a similar way and cosegregation between phenylpropanoid and classical seed coat color genes will be tested.

**P54. *Arabidopsis* Plant U-Box (PUB) E3 ubiquitin ligases have diverse regulatory roles during plant growth and development.**

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The ability of plants to sense and respond to environmental and endogenous signals is essential to their development. Ubiquitin-mediated proteolysis is an important process in regulating responses to environmental or developmental cues. The focus of this research is on PUB-ARM E3 ligases for which a 41-member family exists in *Arabidopsis*. As the U-box E3 family is a recently identified player in this ubiquitination process, little is known about their regulatory roles in plants. So far, roles have been identified in self-incompatibility, plant defense, abiotic stress, and hormone responses. PUB-ARM T-DNA insertion lines were screened for altered growth and with closer inspection of selected lines, phenotypes emerged. *PUB44* lines showed altered growth during seed germination and seedling growth. While *pub44* seedlings grew very poorly, seeds from *pub44/+* plants had altered germination in the presence of ABA. Crosses between insertion lines for *PUB19* and *PUB18* uncovered a distorted segregation ratio. *pub18/pub19* plants were never observed among the F<sub>2</sub> generation and selfed *pub18/+ pub19/+* plant progeny showed a ratio that did not reflect independent gene assortment. Thus, while *PUB44* appears to have a role in regulating cell death and ABA responses, *PUB19* and *PUB18* appear to have a role during gametophytic transmission.

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